

Vol. XV, Part V

October, 1945

THE

IMP. INST.
LIBRARY

18 DEC 1946

AS. 60B

OF

INDIAN JOURNAL OF AGRICULTURAL SCIENCE

Issued under the authority

of

The Imperial Council of Agricultural Research



Annual subscription
Rs. 15 or 23s. 6d.

Price per part
Rs. 3 or 5s.

PUBLISHED BY THE MANAGER OF PUBLICATIONS, DELHI
PRINTED BY THE MANAGER, GOVERNMENT OF INDIA PRESS, CALCUTTA
1946

CONTENTS

VOL. XV, PART V

(October 1945)

The Editorial Committee of the Imperial Council of Agricultural Research, India, takes no responsibility for the opinions expressed in this Journal

Original articles—

PAGE

OBSERVATIONS ON THE INFLUENCE OF NITRATE, AMMONIUM AND IRON ON THE GROWTH AND NUTRITION OF THE RICE PLANT (WITH PLATE VIII AND TWO TEXT-FIGURES)	<i>R. D. Asana</i>	227
STUDIES ON THE VIRUS DISEASES OF POTATOES IN INDIA, II. SOLANUM VIRUS 2 (ORTON.) (WITH PLATES IX & X)	<i>R. Sahai Vasudeva and T. B. Lal</i>	240
THE CYTOLOGY OF <i>CARICA PAPAYA</i> LINN. (WITH PLATE XI AND 53 TEXT-FIGURES)	<i>L. S. S. Kumar, A. Abraham and V. Srinivasan</i>	242
<i>CRYPTOSTEGIA GRANDIFLORA</i> R. BR., A WAR TIME SOURCE OF VEGETABLE RUBBER, V. METABOLIC STUDIES (WITH PLATE XII AND ONE TEXT-FIGURE)	<i>R. H. Siddiqui and S. A. Warisi</i>	254
STUDIES ON THE BORON STATUS OF SOME BENGAL SOILS	<i>M. O. Ghani and A. K. Fazlul Haque</i>	257
SOME OBSERVATIONS ON THE GENETICS OF LINSEED	<i>T. S. Sabnis and T. R. Mehta</i>	263
METHODS OF COMPUTING PEST INCIDENCE (WITH ONE TEXT-FIGURE)	<i>H. S. Pruthi and S. Pradhan</i>	265
SOME FIELD OBSERVATIONS ON THE PRESENT CYCLE OF DESERT LOCUST (<i>SCHISTOCERCA GREGARIA</i> FORSK.)	<i>M. Haroon Khan</i>	270
STUDIES ON <i>EARIAS</i> SPECIES (THE SPOTTED BOLLWORMS OF COTTON) IN THE PUNJAB, IV. THE HOSTS AND HOST-PREFERENCE OF <i>EARIAS CUPREOVIRIDIS</i> WLK., <i>E. FABIA</i> STOLL. AND <i>E. INSULANA</i> BOISD.	<i>M. Haroon Khan, L. R. Mohindra, G. R. Sharma and M. Abdul Ghani</i>	275

Research note—

A PRELIMINARY NOTE ON THE DISTRIBUTION OF FRUIT PULP IN CITRUS SQUASHES	<i>Girdhari Lal and Nagina Lal Jain</i>	281
---	---	-----

Plant Quarantine Notifications		281
--------------------------------	--	-----

ORIGINAL ARTICLES

OBSERVATIONS ON THE INFLUENCE OF NITRATE, AMMONIUM AND IRON ON THE GROWTH AND NUTRITION OF THE RICE PLANT

By R. D. ASANA, Plant Physiologist, Central Sugarcane Research Station, Pusa

(Received for publication on 25 November 1944)

(With Plate VIII and two text-figures)

THERE appears to be a considerable difference of opinion on the relative merits of nitrate and ammonium ions as sources of nitrogen for the rice plant. As the literature on the subject is very extensive no attempt is made to review it; recent investigations which bear directly on the observations reported in this paper are alone considered.

Espino and Estioko [1931], Dastur and Malkani [1933] and Dastur and Pirzada [1934] find a mixture of both forms of nitrogen more satisfactory than either used alone. Thelin and Beaumont [1934] find that in a mixture of nitrate and ammonium nitrogen relatively greater proportion of the former is more satisfactory than the reverse. Gericke [1930] obtained satisfactory growth with nitrate alone as the source of nitrogen in a complete culture solution. Willis and Carrero [1923] from a comparative study of the influence of nitrate and ammonium in soil cultures conclude that the nitrogen of calcium nitrate may be as suitable to the rice plant as that of ammonium in which the reaction of the unassimilated residue does not interfere with the absorption and utilization of iron.

It appears that the earlier work of Gile and Carrero [1920] and Willis and Carrero [1923] did not receive the attention it deserved. They were the first, so far as the writer is aware, to point out the relation of iron to nitrate and ammonium ions. Gines [1930] from Espino's laboratory, did not compare the influence of iron salts on nitrate and ammonium cultures used separately although that is the important point as already made clear by Willis and Carrero [1923]. Dastur and John [1938] investigated the influence of different salts of iron and different pH on the growth of rice seedlings in Knop's solution as modified by Tottingham. Seedlings 14 days old from the time of sowing were used and the experiment lasted for a fortnight. There was no difference between cultures with ferrous sulphate and without iron. The lowest dry weight at pH 3.6 was 233.3 mg. (per 30 plants) as against the highest obtained at pH 7.0, viz. 261.0 mg. Thus in a fortnight only 28 mg. dry weight (per 30 plants) was added which appears to be very low. These workers make no mention of the light and temperature conditions prevailing at the time of the experiment.

Dastur and Malkani [1933] did not repeat their absorption experiments during the 8 to 18 day stages, as well as those on the influence of different concentrations with complete culture solutions; also they did not collect growth or dry matter data from these experiments. Dastur and Pirzada [1934] obtained growth data from the transplantation stage onwards but they applied fertilizers directly to the soil which unfortunately introduced another complication.

It was the aim of this investigation to study more fully the interaction of iron, nitrate and ammonium ions on the growth and nutrition of rice in its early stage, as this aspect had not received enough attention so far. The writer is conscious of the limitations of the data presented but as there is no possibility of the work being continued the observations have been brought together in the hope of calling attention of other workers to this aspect of the problem.

EXPERIMENTAL

Seeds of a pure strain of rice (lowland variety Co 10) obtained from the Coimbatore Paddy Breeding Station were used throughout. The seeds were steeped in water for about six hours and then kept between moist filter paper at room temperature for two or three days until the radicle attained a length of 3-4 mm. The germinated seeds were then transferred to perforated cork mats floating on tap water in glazed porcelain beakers; the seedlings were used for experiment when the shoot had grown about 4 cm. long. The time between soaking the seeds and the shoots attaining the length of about 4 cm. varied from six to nine days depending upon the temperature prevailing at the time of the year; these observations were carefully recorded.

For experiment the plants were kept outside in a wire netting cage (mesh about $\frac{3}{4}$ sq. in.) with a glass roof, which fairly approximated to a green house. Thus the experiments were carried out under natural conditions of light and temperature. Daily maximum and minimum temperature records were kept and the records of hours of bright sunshine were obtained from the local meteorological station about a mile away. Although topographical variations are bound to occur these records throw

some light on the differences in results obtained at different times and these will be considered in the appropriate place.

The culture vessel consisted of a tall glass beaker (resistance glass) of one litre capacity, made opaque to light by thick paper wrappings. On top of the beaker rested a 'urolite' disc with seven perforations to receive the plants, one in each, which were held in position by cotton-wool plugs.

A few experiments were carried out in diffuse light (i.e. not direct sunlight and under shade); this light was, however, not enough for good growth of the plants as results of Experiment 1 show. The culture vessel in these experiments consisted of a glazed porcelain pot about 7 in. diameter and 600 c.c. capacity. These experiments are referred to as 'diffuse light experiments' (Experiments 1 and 2).

In all experiments complete culture solutions were used. The culture solutions with ammonium and nitrate ions as the only sources of nitrogen will henceforth be referred to, for the sake of brevity, as NH_4 and NO_3 cultures respectively. Glass distilled water was always used for making up the cultures. The NH_4 culture had the composition: KH_2PO_4 , MgSO_4 , CaSO_4 , $(\text{NH}_4)_2\text{SO}_4$ and FePO_4 and the NO_3 culture: KH_2PO_4 , MgSO_4 , $\text{Ca}(\text{NO}_3)_2$ and FeSO_4 . The concentration of each nutrient in p.p.m. was: K_2O , 14; P_2O_5 , 21; N, 14; Ca, 19; Mg, 24; and Fe, 8.

Methods of chemical analysis

Drying. The fresh plant material was first dried for about half an hour at about 90°C . and then between 65° and 70°C . for about 27 hours, when the dry weight was found to be constant.

Nitrogen was estimated by the micro-Kjeldahl method as adapted by Parnas and Wagner and described by Pregl [1924]. Nitrate was reduced by reduced iron. Calcium from the plant ash was precipitated as Ca-oxalate by ammonium oxalate at pH 4.5 and the precipitate was then titrated against KMnO_4 . For manganese, all interfering ions including chloride were removed and the magenta colour developed in the filtrate on adding sodium bismuthate was matched against a standard after centrifuging. Iron was estimated by the micro-method described by Straub [1934]. pH was estimated colorimetrically with the 'Hellige comparator' and standard colour discs.

EXPERIMENT 1

The data from this experiment are given in Table I. The experiment was continued for three weeks starting with one-week old seedlings.

TABLE I

Uptake of nitrogen by NH_4 - and NO_3 -plants in diffuse light

	NH_4 series	NO_3 series
Dry weight per 32 plants — shoot .	568.3 mg.	571.6 mg.
" " " " " root .	122.8 mg.	194.8 mg.
" " " " " total .	691.1 mg.	766.4 mg.
Total uptake of N during the experiment	20.14 mg.	17.18 mg.

It will be seen that the NO_3 -plants made as good growth as the NH_4 -plants, the root having made a decidedly better growth in the former. Seedlings (at the fourth leaf stage) from each culture were subsequently set up in one-litre beakers with a view to observing their further growth. Culture solutions of the same strength and composition were used and were renewed at ten day intervals. At the end of the fortnight the first three leaves in the NH_4 -set had withered and before the end of another fortnight all the plants were dead. After three months flowering spikes appeared in the NO_3 -plants. There was no development of tillers in these plants.

EXPERIMENT 2

In order to see whether FePO_4 was a suitable source of iron for nitrate cultures another experiment was carried out with the same nitrate culture solution as used in Experiment 1 but with FeSO_4 and FePO_4 as sources of iron. FePO_4 was prepared according to Livingstone's procedure as described by Gines [1930]. The experiment was continued for 20 days. Chlorosis developed in the FePO_4 series after about twelve days, thus indicating that the iron from FePO_4 , which was in colloidal form, was not readily available to the plants. Similar results have been recorded by Jones and Shive [1921]. The total dry weight produced and the total uptake of nitrogen were both less in the FePO_4 treatment.

It will thus be seen that even in diffuse light it is possible to grow rice up to the flowering stage, provided care is taken to supply iron in a suitable form. That rice does not thrive in NH_4 cultures during later stages is also evident. In view of the above finding it is surprising that Macasaet [1936] working in Espine's laboratory, should state: 'But no culture has yet been found that could bring the rice plant to complete maturity. Espino [1920] and Espino and Estioko [1931] succeeded in growing rice plant normally for the first thirty or forty days in certain culture solutions. Beyond that stage of development, however, the plant always produced chlorotic young leaves.'

It may also be noted that Gericke [1930] succeeded in growing plants to maturity in NO_3 cultures with ferric tartarate as the source of iron.

It will be noted from Experiment 1 that the absorption rates of both NO_3 and NH_4 nitrogen are remarkably low. Such low absorption rates have also been obtained by Dastur and Malkani [1933]. The slow growth rate of the plants under low light intensity (diffuse light) would seem to account for these low absorption rates. In order to see what kind of growth curve obtained under these conditions, during early stages, an experiment was carried out in which dry weight data were collected every two days. Seedlings about a week old were used to begin with (4/10/37) and the nitrate culture was used. In Fig. 1 is shown the curve of increase in dry weight in mg. per five plants (mean of 2 replicates). Mean temperature (mean of 2 maximum and 2 minimum temperatures) during each interval is also shown in Fig. 1.

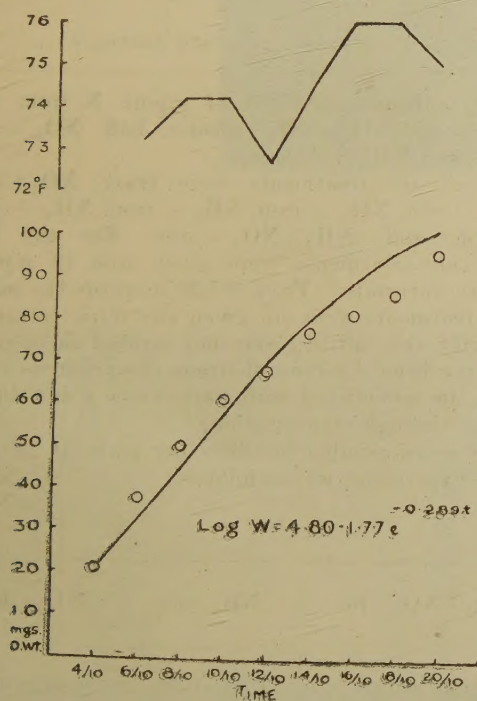


FIG. 1. Increase in dry weight in diffuse light

It will be seen that the growth curve is of a decrement type in which the growth rate is continuously falling with time. Similar growth curves have also been obtained by Gregory [1928] for the surface growth of cotyledons and leaves of cucumber in artificial light at supra-optimal temperatures and by White [1936] for the growth of *Lemna* under potassium deficiency in the culture medium. White

[1936] has fitted to this type of growth curve an equation of the type, $\frac{1}{n} \cdot \frac{dn}{dt} = re^{-kt}$, which assumes that the growth rate itself is falling exponentially with time due to an inhibiting factor, which in our case would seem to be low light intensity. The smooth curve shown in Fig. 1 is based on the equation

$\log \frac{n}{n_0} = \frac{r}{k} (1 - e^{-kt})$ and it will be seen that the agreement is moderately close. This observation might account for the very small amount of growth observed by Dastur and John [1938] during the second fortnight from the time of sowing during which they carried out their experiment and for the low rate of nitrogen uptake as reported by Dastur and Malkani [1933]. Although neither Dastur and John [1938] nor Dastur and Malkani [1933] mention what light conditions prevailed during their experiments, it would seem, judging from their absorption rates and growth data, that their experiments were carried out under sub-optimal light conditions.

EXPERIMENTS IN SUNLIGHT

With a view to studying the growth and nitrogen uptake rates obtaining at a higher light intensity an experiment was carried out in the cage, exposed to sunlight as already described.

EXPERIMENT 3

In this experiment both growth rates and uptake of nitrogen were measured. Weather throughout the experimental period was very clear and the experiment was continued for about three weeks. Both dry weights and mean temperature are plotted in Fig. 2. The curve of dry weight increase, as given in Fig. 2, conforms very well with that of an exponential type and agreement with the smooth curve is very good considering that no attempt was made to control the environment. The curve of daily uptake of nitrogen approximately followed the dry weight curve, shown in Fig. 2, and the total uptake in 18 days was 191.08 mg. per 25 plants.

With a view to observing further growth in sunlight a few plants were transferred to one litre beakers with the same culture solution renewed periodically. These plants tillered well and flowered after about 14 weeks.

With a view to elucidating the interrelations between Fe, NH_4 and NO_3 ions experiments on a more elaborate scale were carried out. Tall beakers, one litre in capacity carrying seven plants each, were used. Culture solutions of higher concentration were used so that they would be required to be renewed once a week only. The composition of the

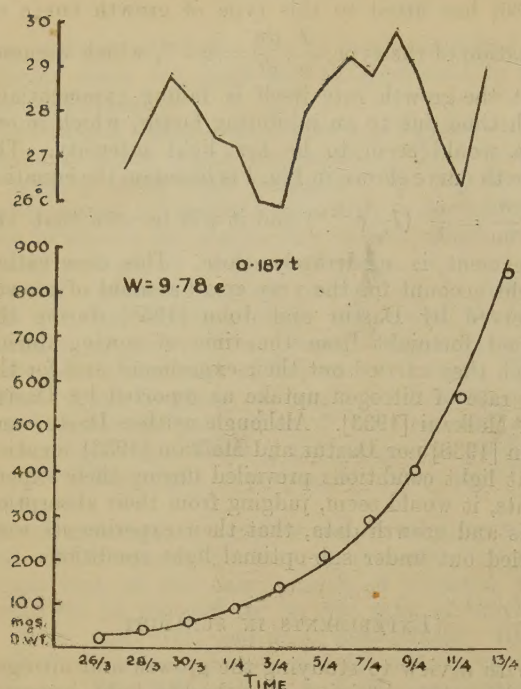


FIG. 2. Increase in dry weight in sunlight

three culture solutions used from time to time is given below :—

NO ₃ culture	NH ₄ culture	NH ₄ + NO ₃ culture
Ca (NO ₃) ₂	(NH ₄) ₂ SO ₄	Ca(NO ₃) ₂ + (NH ₄) ₂ SO ₄
K ₂ SO ₄ KH ₂ PO ₄ Mg SO ₄	K ₂ SO ₄ KH ₂ PO ₄ Mg SO ₄ Ca SO ₄	K ₂ SO ₄ KH ₂ PO ₄ Mg SO ₄ Ca SO ₄

NO ₃ + Fe	NO ₃ — Fe	NH ₄ + NO ₃ + Fe	NH ₄ + NO ₃ — Fe	NH ₄ + Fe	NH ₄ — Fe
2.75 ± 0.13	1.08 ± 0.08	2.60 ± 0.16	3.25 ± 0.13	1.50 ± 0.15	2.25 ± 0.18

Senescence set in very rapidly in NH₄ plants starting from the leaf tips. The root system in the NO₃ plants was long, white and healthy. In about a week after starting the experiment it was noticed that the secondary roots of both NH₄ and NH₄ + NO₃ plants ceased to grow in length. Later on a thick bunch of root initials surrounded the base of the

Boron and manganese were always added to each culture solution in concentration of 1 p.p.m.

EXPERIMENT 4

Seeds soaked on 20/4/38. Seedlings were planted in apparatus on 26/4/38. Concentrations of culture solutions as used from time to time were as below :

Date	Mg	Ca	P ₂ O ₅	K ₂ O	N	Fe
26/4-3/5	24	60	12.3	25.0	42	8 p.p.m.
3/5-10/5	24	60	12.3	25.0	42	8 „
10/5-13/5	24	60	12.3	25.0	42	8 „
13/5-16/5	24	60	12.3	25.0	42	8 „
16/5-23/5	24	180	24.6	67.0	126(NO ₃)	8 „

NH₄ cultures contained 84 p.p.m. N from 16/5 to 23/5 and NO₃ + NH₄ cultures had NO₃ = 84 p.p.m. and NH₄ = 42 p.p.m.

In all six treatments were tried : NO₃ + iron, NO₃ — iron, NH₄ — iron, NH₄ + iron, NH₄ + NO₃ + iron and NH₄ + NO₃ — iron. For the first week all treatments were given iron (8 p.p.m.) as ferric tartarate. From 3/5/38 onwards the minus iron treatments were not given any ferric tartarate. Although the cultures were not aerated there could not have been a serious shortage of oxygen as these had to be replenished with water twice a day due to its loss through transpiration.

The mean number of tillers per plant at the end of the experiment was as follows :

shoot in the NH₄ plants which did not elongate. The NH₄ + NO₃ plants had a thick bunch of white adventitious roots which were quite healthy, while those of NH₄ plants were brown and unhealthy. Although the dry weights were the same the character of the root system in NH₄ + NO₃ plants was strikingly different (Plate VIII).

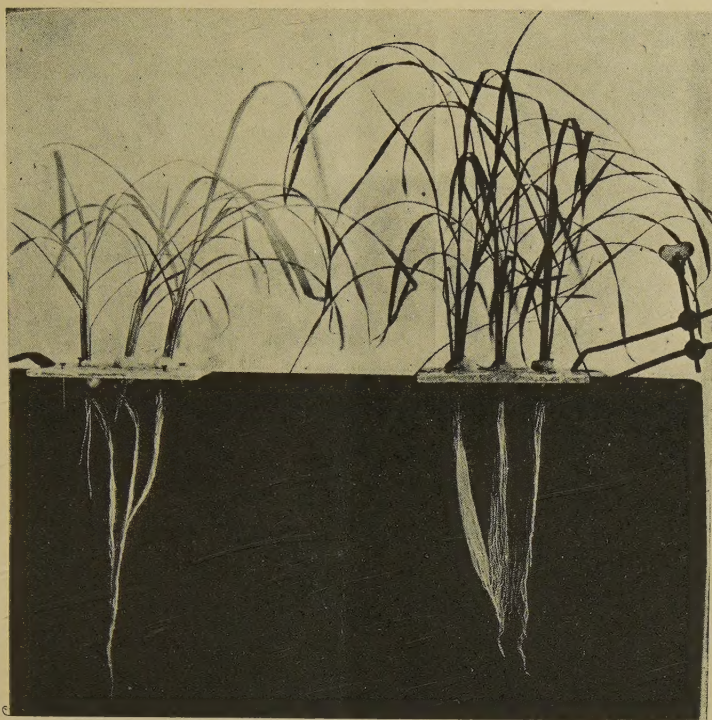


FIG. 1.

$\text{NO}_3\text{-Fe (pH 6.0)}$

$\text{NO}_3\text{+Fe (pH 6.0)}$



FIG. 2.

$\text{NH}_4\text{-Fe (pH 4.7)}$

$\text{NH}_4\text{+Fe (pH 4.7)}$



FIG. 3.

$\text{NH}_4\text{+NO}_3\text{-Fe (pH 4.7)}$

$\text{NH}_4\text{+NO}_3\text{+Fe (pH 4.7)}$

TABLE II

Mean dry weights in gm. (d.w.) and water content as percentage of dry weight (w.c.) per sample of 6 plants (mean of two replicates) on 23/5/38

	Leaf	Sheath	Shoot	Root	Total
NH ₄ + NO ₃ + Fe	2.9631±	2.0979±	5.0612	0.9231±	5.9843±
d.w.	0.1124	0.0199	..	0.0547	0.1472
w.c.	228	487	..	787	..
NH ₄ + NO ₃ — Fe	2.9286±	2.0114±	4.9400	0.9122±	5.8522
d.w.	0.2244	0.1687	..	0.0673	0.4604
w.c.	226	510	..	786	..
NO ₃ + Fe	2.6889±	1.8114±	4.5003	0.8725±	5.3729±
d.w.	0.0179	0.0348	..	0.0263	0.0790
w.c.	233	544	..	749	..
NO ₃ — Fe	1.3664±	0.6654±	2.0318	0.3990±	2.4308±
d.w.	0.1103	0.0595	..	0.0239	0.1937
w.c.	273	750	..	751	..
NH ₄ + Fe	1.3580±	0.9918±	2.3498	0.2823±	2.6321±
d.w.	0.033	0.0164	0.019	0.0219	0.0723
w.c.	185	460	..	625	..
NH ₄ — Fe	1.3641±	1.1561±	2.5202	0.1961±	2.7163±
d.w.	0.099	0.1023	..	0.0034	0.0967
w.c.	181	375	..	626	..

Although the dry weights of each plant part of both NH₄ + NO₃ series is higher than that of NO₃ + Fe, the difference does not attain the level of statistical significance which, however, might be due to the small number of replicates. Only NO₃ plants are markedly influenced by iron deficiency. The NH₄ plants have a lower water content. The rates of nitrogen uptake differ approximately in the same direction as the dry weights in different treatments. It may also be noted that the rates of uptake of NO₃ and NH₄ ions are almost the same in

the early stages, which is quite contrary to the observations of others. It must be remembered that the differences during the last week (16/5-23/5) are due to the differences in the original concentrations of the respective ions.

The iron contents in mg. per gm. dry weight of leaf are given in Table IV. It will be seen that the iron content of NO₃—Fe and NH₄—Fe plants is approximately the same in spite of which the leaves in the former series (except the first four leaves) were chlorotic, while those in the latter remained

TABLE III

Mean uptake of nitrogen in mg. per seven plants (mean of two replicates)

Date	NO ₃ + Fe	NO ₃ — Fe	NH ₄ + NO ₃ + Fe	NH ₄ + NO ₃ — Fe	NH ₄ + Fe	NH ₄ — Fe
6/4-3/5	9.11	9.26	6.84 + 6.83	6.55 + 5.55	8.71	10.25
5-10/5	32.74	27.90	20.35 + 20.78	20.07 + 20.21	21.53	24.43
10-13/5	28.70	16.08	18.51 + 17.07	16.37 + 18.36	20.01	23.42
13-16/5	40.71	19.50	20.20 + 20.08	20.63 + 19.79	20.5	28.7
16-23/5	116.75	49.10	44.42 + 72.30	43.99 + 77.61	51.67	71.74
Total	228.01	121.84	247.38	249.13	122.42	158.54

TABLE IV

The iron content in mg. per gm. dry weight of leaf

NO ₃ + Fe	NO ₃ — Fe	NH ₄ + NO ₃ + Fe	NH ₄ + NO ₃ — Fe	NH ₄ + Fe	NH ₄ — Fe
0.34	0.1893	0.4675	0.3532	0.4179	0.1772

green. The iron content of $\text{NH}_4 + \text{NO}_3 - \text{Fe}$ plants which was as high as that of $\text{NO}_3 + \text{Fe}$ plants might perhaps be accounted for by the lower $p\text{H}$ of the culture solution which might have dissolved more iron during the first week and subsequently from the impurities of the salts. It might be argued that the $\text{NH}_4 + \text{NO}_3$ plants did not really suffer from iron deficiency and therefore the question whether these plants can really do with smaller amount of iron still remains open.

EXPERIMENT 5

Seeds were soaked for about six hours on 8/6/38 and seedlings were placed in apparatus on 15/6/38. Treatments were as under, replicated three times:

(1) $\text{NO}_3 + \text{Fe}$ ($p\text{H}$ 4.7); (2) $\text{NO}_3 + \text{Fe}$ ($p\text{H}$ 6.0); (3) $\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 6.0); (4) $\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 4.7). $p\text{H}$ 4.7 was maintained by adding required amount of H_2SO_4 .

Strengths of the culture solutions are given below:

15/6—22/6. Same as in Experiment 4 for the first week.

22/6—29/6. Same as in Experiment 4 for the first week.

29/6—6/7. $\text{N} = 84$; $\text{K}_2\text{O} = 42$; $\text{P}_2\text{O}_5 = 12.3$; $\text{Ca} = 120$; $\text{Mg} = 48$; $\text{Fe} = 8$ p.p.m.

6/7—13/7. $\text{N} = 105$; $\text{K}_2\text{O} = 44.0$; $\text{P}_2\text{O}_5 = 15.0$; $\text{Ca} = 150$ p.p.m.

Other elements were as used during the first two weeks. Iron as ferric tartarate, was given to all the four series for the first week, after which it was omitted from the deficient series. After about 12 days, the leaves of $\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 4.7) set folded inwards along their midribs and plants from two replicate beakers died off. Plants in the third replicate recovered and afterwards they grew fairly well, but on account of this early setback they cannot strictly be compared with the other treatments. The noteworthy features of these plants were that they did not become chlorotic, while the leaves of the $\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 6.0) plants developed chlorosis from the third leaf onwards. The root system in both $\text{NO}_3 + \text{Fe}$ ($p\text{H}$ 4.7) and $\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 4.7) plants lagged behind those in the other treatments in development during the first week but later many adventitious roots grew out and presented the same appearance as the roots of the $\text{NH}_4 + \text{NO}_3$ plants of Experiment 4.

TABLE V

Mean dry weight in gm. per sample of six plants on 13/7/38

(Mean of three replicates)

$\text{NO}_3 + \text{Fe}$ ($p\text{H}$ 4.7)	$\text{NO}_3 + \text{Fe}$ ($p\text{H}$ 6.0)	$\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 6.0)	$\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 4.7)
2.7674 ± 0.1370	3.6756 ± 0.0572	1.8973 ± 0.1430	1.8202

The difference due to $p\text{H}$ in the $\text{NO}_3 + \text{Fe}$ treatments is significant. It is not clear whether lower $p\text{H}$ or greater uptake of iron is responsible for this depressing effect.

The rates of uptake of nitrogen show the same differences as the dry weights. As from the second week onwards the minus iron plants at $p\text{H}$ 4.7 began

to dry up and subsequently died, the rate of uptake was very slow in the second week. If we compare, however, the rates during the first week when the treatments were identical, except the differences in $p\text{H}$, we see that $p\text{H}$ had little influence on the rate of uptake of nitrate nitrogen. At $p\text{H}$ 4.7 it was 14.54 and at $p\text{H}$ 6.0, 14.96.

TABLE VI

Mean rate of uptake of nitrogen in mg. by seven plants

	$\text{NO}_3 + \text{Fe}$ ($p\text{H}$ 4.7)	$\text{NO}_3 + \text{Fe}$ ($p\text{H}$ 6.0)	$\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 6.0)	$\text{NO}_3 - \text{Fe}$ ($p\text{H}$ 4.7)
15/6—22/6 . . .	5.47	8.20	6.76	9.07
22/6—29/6 . . .	17.70	23.17	16.41	4.03
29/6—6/7 . . .	42.39	51.39	29.80	not estimated
6/7—13/7 . . .	54.71	69.11	30.64	do.
Total . . .	120.27	151.87	83.64	..

TABLE VII
Iron content in mg. per gm. dry weight

	NO ₃ +Fe (pH 4·7)	NO ₃ +Fe (pH 6·0)	NO ₃ -Fe (pH 6·0)	NO ₃ -Fe (pH 4·7)
Leaf	0·706	0·407	0·340	0·285
Sheath	0·493	0·248	0·314	0·280

In this experiment the iron content is much higher in both NO₃+Fe (pH 6·0) and NO₃-Fe (pH 6·0) plants as compared to the identical series of Experiment 4. It is probable that this very high content is due to much less growth made in this experiment. Although the leaves in the NO₃+Fe (pH 4·7) plants were a shade deeper green their efficiency in dry matter production was not evidently increased.

EXPERIMENT 6

Seeds were soaked on 13/7/38 and seedlings set up in apparatus on 20/7/38. Treatments were as under, each having been replicated thrice:

NO₃+Fe (pH 6·0); NO₃-Fe (pH 4·7); NO₃-Fe (pH 6·0); NH₄+Fe (pH 4·7); NH₄-Fe (pH 6·0). During the first week all treatments were given iron as ferric tartarate and also all were maintained at pH 4·7 except NO₃+Fe; from the second week onwards iron was omitted from the deficiency series and the pH values shown in brackets were maintained by daily checking and adding requisite amounts of sulphuric acid or caustic soda. The idea in keeping the pH at 4·7 in all treatments except NO₃+Fe during the first week was this: at pH 6·0 and higher than this iron tends to precipitate gradually and the roots are covered with a brown insoluble deposit of iron oxides, thus making

the chemical analysis of roots for iron impossible. The other object was to keep the concentration of iron in the two NO₃-Fe cultures identical during the first week so that the plants can have the same total iron content. It must, however, be recorded that the shift in pH during the period of 24 hours was quite large, that of NH₄-Fe (pH 6·0) decreasing by about 1·5 units and that of NO₃-Fe (pH 4·7) increasing by about 1·3 units. In spite of this incomplete control of pH, the differences obtained were sufficiently striking, although experiments, under better controlled conditions, would certainly seem to be more desirable. Out of three replicates under each treatment one was reserved for the checking of pH. The other two were used for the estimation of the uptake of nitrogen. Although the replicate used for the estimation of pH was deprived daily of its culture solution by 10 to 20 c.c., the deficit thus caused could not be very appreciable because the concentration of each element was sufficiently large. The concentrations of the culture solutions used were as under:

20/7—27/7	. Same composition as in Experiment 4 for the NO ₃ and NH ₄ treatments
27/7—3/8	. Same composition as in Experiment 4 for the NO ₃ and NH ₄ treatments
3/8—10/8	. N = 84; K ₂ O = 42; P ₂ O ₅ = 12·3 p.p.m.
10/8—17/8	. N = 105; K ₂ O = 44·0; P ₂ O ₅ = 15·0 p.p.m.

TABLE VIII

Mean dry weight in gm. (d.w.) per six plants (mean of three replicates) and water content as percentage of dry weight (w.c.) on 17/8/38. Standard errors in NO₃-Fe (pH 4·7) and NO₃-Fe (pH 6·0) could not be estimated because the first three and the remaining leaves were weighed separately. As the sample for the first three leaves was very small the three replicates were weighed together.

	Leaf	Sheath	Shoot	Root	Total
NO ₃ +Fe (pH 6·0)	1·9874±	1·5786±	3·5660±	0·9224±	4·4884±
d.w.	0·109	0·1711		0·0791	0·2592
w.c.	251	522		796	
NO ₃ -Fe (pH 4·7)	1·7824	1·4298	3·2122	0·9281	4·1403
d.w.	265	575		942	
NO ₃ -Fe (pH 6·0)	0·7571	0·4587	1·2098	0·2864	1·4962
d.w.	264	652		960	
NH ₄ +Fe (pH 4·7)	1·2519±	1·1452±	2·3971	0·2249±	2·6220±
d.w.	0·04	0·1576		0·01403	0·2108
w.c.	176	354		631	
NH ₄ -Fe (pH 6·0)	1·5829±	0·9508±	2·5338	0·4069±	2·9407±
d.w.	0·2444	0·2095		0·0346	0·4784
w.c.	257	647		915	

The marked effect of higher pH is indicated by the increased dry weight of the root system of NH_4 —Fe (pH 6.0) plants. One might, therefore, conclude with some justification that it is the low pH that inhibits root development rather than NH_4 ion *per se*. Not only has the dry weight increased but also the water content in every part of the plant. The individual leaves also died off more slowly than those of the plants grown at pH 4.7. Another remarkable feature was that no chlorosis developed in the leaves in spite of deficiency of iron, while it did in the case of NO_3 —Fe (pH 6.0) plants (from the fourth leaf onwards). Chlorosis did not develop in the NO_3 —Fe (pH 4.7) plants and they made almost as good growth as the NO_3 +Fe (pH 6.0) plants. The NO_3 —Fe (pH 4.7) plants were also characterized by a short root system with a thick

bunch of short adventitious roots of the type that developed in the NO_3 + NH_4 plants of Experiment 4 and NO_3 +Fe (pH 4.7) plants of Experiment 5. Evidently there was better utilization of iron in this case.

The rates of uptake of nitrogen followed the same trend as the dry weights. As has already been pointed out, during the first week all the treatments, except NO_3 +Fe, were maintained at pH 4.7. It will be seen that at pH 4.7 there was no greater uptake of nitrate nitrogen than at pH 6.0. Similarly during the first three weeks there was little difference in the uptake of ammonium at pH 4.7 and 6.0 respectively. Later on the plants at pH 6.0 developed better root system and also their leaves did not age as quickly and hence we find a greater uptake of ammonium.

TABLE IX

Mean rate of uptake of nitrogen in mg. per seven plants

	NO_3 +Fe (pH 6.0)	NO_3 —Fe (pH 6.0)	NO_3 —Fe (pH 4.7)	NH_4 +Fe (pH 4.7)	NH_4 —Fe (pH 6.0)
20/7—27/7	7.05	6.16	7.05	5.90	7.46
27/7—3/8	22.74	14.10	17.85	21.42	16.58
3/8—10/8	63.33	33.40	56.43	48.53	48.87
10/8—17/8	119.62	37.13	114.87	52.51	79.11
Total	212.74	90.79	196.20	128.38	152.02

TABLE X

Iron content in mg. per gm. dry weight

Treatment	Leaf	Sheath	Root
NH_4 +Fe (pH 4.7)	0.465	0.316	..
NH_4 —Fe (pH 6.0)	0.262	0.250	..
NO_3 +Fe (pH 6.0)	0.395	0.383	..
NO_3 —Fe (pH 6.0)	0.456 (chlorotic)	0.187	1.88
	0.427 (green)	0.456	..
NO_3 —Fe (pH 4.7)	0.329 (upper)	0.273	0.701
	0.589 (lower 3)	0.694	..

In the NO_3 —Fe (pH 6) leaves the iron content was higher than that of the NO_3 +Fe ones. Although the iron content of NO_3 —Fe (pH 4.7) leaves was lower than that of the NO_3 —Fe (pH 6) ones there was no chlorosis in the former. Although the NH_4 —Fe (pH 6) leaves contained much less iron than the NH_4 +Fe ones they did not develop chlorosis.

The calcium content of NH_4 -leaves was markedly less than that of the NO_3 ones. At lower pH the Ca content was much less in both NO_3 and NH_4 leaves.

Striking differences in the manganese* content of the plants of different treatments were also

* Manganese could not be estimated quantitatively for want of time. The differences were judged only qualitatively.

found. The NH_4 -plants were characterized by the lowest content of manganese. The highest content was found in the first three green leaves of the NO_3 -Fe (pH 6) plants, followed by the chlorotic leaves of the same.

TABLE XI

Calcium (as CaO) content as percentage of dry weight of leaves

Treatment	Leaf
NH_4 -Fe (pH 4.7)	0.428
NH_4 -Fe (pH 6.0)	0.710
NO_3 -Fe (pH 6.0)	1.512
NO_3 -Fe (pH 4.7)	1.125

DISCUSSION

A. Influence of light and temperature

We have already noted the differences in the type of growth curves obtained in diffuse and sunlight respectively (Experiments 3 and 4). We may now briefly refer to the influence of this factor as well as temperature on the other experiments for which the relevant data are given in Table XII.

TABLE XII

Influence of light and temperature on experiments

Experiment	Treatment	Mean dry weight	Mean hours bright sunshine per day	Mean temperature
1	NO_3 -Fe (pH 6)	5.3729 gm.	8.9	86.8°F.
	NO_3 -Fe (pH 6)	2.4308 ..	8.9	86.8°F.
5	NH_4 -Fe (pH 4.7)	2.6321 ..	8.9	86.8°F.
	NO_3 -Fe (pH 6)	3.6756 ..	3.9	78.4°F.
6	NO_3 -Fe (pH 6)	1.8973 ..	3.9	78.4°F.
	NO_3 -Fe (pH 6)	4.4884 ..	4.9	78.7°F.
	NO_3 -Fe (pH 6)	1.4962 ..	4.9	78.7°F.
	NH_4 -Fe (pH 4.7)	2.6220 ..	1.9	78.7°F.

The influence of both light and temperature is evident in the case of NO_3 -Fe and NO_3 -Fe treatments. The reason why the NO_3 -Fe plants of Experiment 6 produced less dry weight than those of Experiment 5, in spite of one more hour of bright sunshine per day, may be that the mean hours of bright sunshine during the first two weeks were 2.6 and 4.4 hours respectively as against 3.9 and 6.9 hours during Experiment 5. Later, although the light conditions improved the plants could not possibly make up because of iron deficiency. The NH_4 -plants, on the other hand, do not show any influence of light and temperature and it is not possible to account for this result in the absence of more data.

As we have already seen that the dry weights and the total rates of nitrogen uptake vary in the same direction, it would be interesting to compare the weekly rates of nitrogen uptake with the weekly means of hours of bright sunshine per day (Table XIII).

It will be seen that there is a close correlation in the case of NO_3 -Fe and NO_3 -Fe treatments only. It is not suggested that light intensity influences the absorption process. The latter is influenced through the acceleration of the growth rate by a higher light intensity.

B. Iron chlorosis

The percentage iron content of chlorotic leaves has been found to be nearly the same as or lower than that of green leaves and it is generally held that iron exists in an insoluble and therefore unavailable form in chlorotic leaves. Indeed, sap analyses have invariably shown that green leaves contain more soluble iron than chlorotic leaves. Olsen [1935] found very high total phosphorus

TABLE XIII

Comparison of weekly rates of nitrogen uptake and weekly means of hours of bright sunshine per day

	1st week	2nd week	3rd week	4th week
Hours of bright sunshine	8.6	9.3	8.8	9.0
NO_3 -Fe (Experiment 4)	9.11	32.74	69.41	116.75
NO_3 -Fe	9.26	27.90	35.58	49.10
NH_4 -Fe	8.71	21.53	40.51	51.67
Hours of bright sunshine	2.6	4.4	7.0	5.9
NO_3 -Fe (Experiment 6)	7.05	22.74	63.33	119.62
NO_3 -Fe	6.16	14.10	33.40	37.13
NH_4 -Fe	5.90	21.42	48.53	52.51
Hours of bright sunshine	3.9	6.9	3.2	1.6
NO_3 -Fe (Experiment 5)	8.2	23.17	51.39	69.11
NO_3 -Fe	6.76	16.41	29.80	30.64

content associated with chlorotic leaves and he held that iron was, therefore, precipitated as ferric phosphate. Since phosphorus exists in the leaves in several forms and combinations, Olsen's suggestion would seem to be far too simple. Wadleigh, Robbins and Beckenbach [1937] believed that their observation of an association between high soluble phosphorus content (in sap) and chlorosis accorded with Olsen's suggestion; this, however, is not evidently true. Chapman [1931] suggested that manganese by stimulating oxidase action converts iron into an insoluble ferric form. This does not seem to account for chlorosis observed in the present work as manganese was found to be in much higher concentration in the first three green leaves of chlorotic plants. Ingalls and Shive [1931] observed that there was a direct relationship between hydrogen ion concentration and soluble iron content in tissue fluids. Chapman [1931], Oserkowsky [1932] and Wadleigh, Robbins and

Beckenbach [1937], however, did not find any such relation in chlorotic-leaves. With a view to testing this point the pH of sap of plants of Experiment 6 was estimated by means of 'Wulff pH tester'. In a few drops of the extracted sap a small strip of indicator foil was dipped for about a minute and the colour developed in the strip matched with standard colour strips. The results are given in Table XIV. It will be seen that there is no association between chlorosis and pH. It appears that although other workers have found a much higher base content associated with chlorosis, they have not regarded this as of any significance. From an inspection of the data of Wadleigh and Shive [1939] and Olsen [1935] on corn grown in nitrate culture it appears that the total base content and soluble iron content are negatively correlated. The percentage ash content of different plant tissues from Experiment 6 also show the same trend (Table XV).

TABLE XIV
pH of sap of plants of Experiment 6

pH of culture solution	Treatment	Tissue	pH of sap
6.0	NO ₃ +Fe	Leaf	6.2
4.7	NH ₄ +Fe	"	5.6
4.7	NO ₃ -Fe	"	6.0
6.0	NH ₄ -Fe	"	6.2
6.0	"	Root	6.2
6.0	NO ₃ -Fe	"	6.2
6.0	"	Leaf	6.2

TABLE XV
Percentage ash content of different plant tissues from Experiment 6

Treatment	pH	Leaf	Sheath	Root
NH ₄ +Fe	4.7	6.6	4.0	..
NH ₄ -Fe	6.0	8.7	9.2	..
NO ₃ +Fe	6.0	6.4	6.6	..
NO ₃ -Fe	6.0	12.9 (chlorotic)	14.0	14.7
"	6.0	11.2 (green)	5.1	..
"	4.7	6.5	7.9	5.7
"	4.7	4.0 (first three)	1.0	..

The total uptake of ash by $\text{NO}_3\text{—Fe}$ (pH 6.0) plants amounted to 190.6 mg. per six plants and by $\text{NO}_3\text{—Fe}$ (pH 4.7) plants to 270.6 mg. whereas the total dry weights were 1.49 and 4.14 gm. respectively. In Table XVI is shown the distribution of iron in plants from Experiment 6. At pH 4.7 distinctly higher amount of iron has been absorbed and this might have been supplied from impurities in the culture solution remaining dissolved at the lower pH. It may be noted that 55 per cent of the total iron content was retained in the roots of plants at pH 6.0 as against 38 per cent at pH 4.7. The differences in the sheath are similarly wide. It appears as if iron is not transferred as rapidly from roots at pH 6.0 as from those at pH 4.7. It is worth recording that subsequent to the change in pH from 4.7 to 6.0 after the first week of the experiment the roots elongated considerably more within 48 hours. How this root behaviour is associated with

the retention of a larger proportion of iron in the root is difficult to visualize. Is it possible that the relatively larger absorption of other ash constituents at pH 6.0 interfered with the transport of iron? The data presented in Table XVII bring out this point more clearly. It will be seen that the water content per unit quantity of ash is considerably less in $\text{NO}_3\text{—Fe}$ (pH 6.0) leaves. The question may well be raised as to why greater uptake of ash did not take place in the $\text{NO}_3\text{+Fe}$ -plants maintained throughout at pH 6.0. With the data at hand it is difficult to answer this question. It can only be surmised that pH 6.0 *per se* cannot have stimulated the uptake of ash by $\text{NO}_3\text{—Fe}$ -plants. The question whether better utilization of iron could occur at the lower pH is also not sufficiently answered by the data at hand. Experiments with highly purified salts and direct estimation of iron in such a culture medium can settle this point.

TABLE XVI

Distribution of iron in plants from Experiment 6

$\text{NO}_3\text{—Fe}$ (pH 6.0)			$\text{NO}_3\text{—Fe}$ (pH 4.7)		
	Absolute iron content in mg.	Percentage of total		Absolute iron content in mg.	Percentage of total
First three green leaves . . .	0.07122	4.3	First three green leaves . . .	0.09200	5.0
Chlorotic leaves	0.26643	29.9	Remaining green leaves . . .	0.53500	32.0
Sheath (chlorotic)	0.06674	6.8	Sheath (remaining leaves) . .	0.36127	21.0
Sheath (green leaves)	0.04641	4.0	Sheath (first three green leaves)	0.07384	4.0
Root	0.53845	55.0	Root	0.65061	38.0
	0.98925	100.0		1.71272	100.0

TABLE XVII

Water, iron and ash contents of leaf

	$\text{NO}_3\text{—Fe}$ (pH 6.0)	$\text{NO}_3\text{—Fe}$ (pH 4.7)	$\text{NO}_3\text{+Fe}$ (pH 6.0)
Water content in mg. per unit leaf area in sq. in.	11.67	16.09	17.70
Iron content in γ^* per unit leaf area in sq. in.	1.85	1.94	2.70
Ash in mg. per unit leaf area in sq. in.	0.52	0.38	0.45
Water content in mg. per mg. ash	22.20	42.00	39.30

 γ^* (gamma) is equal to 0.001 mg.

The data on the first three green leaves of the $\text{NO}_3\text{—Fe}$ (pH 6.0) and on the corresponding ones of the pH 4.7 treatments are interesting and are presented in Table XVIII.

TABLE XVIII

Iron, water and ash contents of the first three green leaves of $\text{NO}_3\text{—Fe}$ (pH 6.0) and (pH 4.7)

	$\text{NO}_3\text{—Fe}$ (pH 6.0)	$\text{NO}_3\text{—Fe}$ (pH 4.7)
Iron content in γ per unit area (sq. in.)	10.66	13.73
Water content in mg. per unit area („)	41.90	42.26
Ash content in mg. per unit area („)	2.80	0.986
Dry weight in mg. per unit area („)	23.20	24.97

Again the difference in ash content is very striking. This higher uptake of ash must have occurred after the first week of the experiment when the pH was raised to 6.0. Presumably iron existed in a soluble and available state during the first week with the

STUDIES ON THE VIRUS DISEASES OF POTATOES IN INDIA

II. SOLANUM VIRUS 2 (ORTON.)

By R. SAHAI VASUDEVA, Seed Potato Production Officer, and T. B. LAL, Assistant, Section of Mycology and Plant Pathology, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 28 October 1944)

(With Plates IX and X)

SOLANUM VIRUS 2 (Potato virus Y, Smith) was recovered during analytical work on potato viruses from potato plants of Phulwa variety showing either negligible mosaic and veinal necroses or severe mosaic only. The virus was also cultured from plants of Gola, Majestic, President, Windsor Castle and Talisman (Up-to-date) varieties showing mosaic and veinal necroses followed by acropetal necrosis. All the varieties excepting certain Phulwa plants showing negligible mosaic and veinal necroses yielded a mixture of Solanum viruses 2 and 1. The composite nature of diseases in these varieties was indicated by specific reactions on certain plant indicators. The separation of Solanum virus 2 from the mixture was effected by passage of the complex through *Petunia hybrida*.

The symptoms exhibited by potato plants of some of the varieties from which Solanum virus 2 was isolated are described below.

Phulwa. The virus in this variety was isolated from plants showing two different types of symptoms, i.e. negligible mosaic with veinal necroses and severe type of mosaic. In the former case the growth of plants is seldom stunted. The plants appear normal and it is hard to read the mosaic symptoms on them. Veinal necroses is present on the under surface of the leaflets. Necroses may commence on the midrib or the minor veins and often reach the petiole, though the occurrence of leaf-drop streak has not so far been observed. Leaves affected with veinal necroses show palish streaks along the midrib and the veins on the upper surface indicating corresponding veinal necroses on the under surface. The texture of the leaves is unaltered but the leaf apex and sometimes even the leaf margins tend to point downwards. The general colour of the foliage of the affected plants, except those showing severe symptoms of infection, is not appreciably changed and the disease may, at times, pass off unnoticed failing a careful examination. Plate IX, fig. 1 shows a Phulwa plant leaf with veinal necroses.

In the case of severe types of mosaic there is intense mottling of the leaf surface. Palish or yellowish areas are visible all over the leaf surface except in the centre where a small patch of green may persist. Sometimes the whole leaf may turn yellowish. The plants are stunted and the leaflets are greatly reduced in size, show distortion and puckering, and develop

purple colour. The general appearance of the foliage is extremely pale. Vein bending is frequently observed and may at times be very prominent. The leaflets are wrinkled, thick, at times almost saucer-shaped and brittle. Necrosis is absent.

Gola. Plants are stunted and assume an erect habit. The upper leaves show negligible or mild mottle, reduction in size, thickening and some distortion. Lower leaves are not appreciably reduced in size. The leaflets are thick and brittle and their tips point in a downward direction. Brown or dark brown veinal necroses are observed on the under surface of leaves. In severe cases the necroses may extend to the petiole and result in leaf-drop streak. Later on the necroses may be visible on the upper surface of leaves. Some leaves may even show necrotic spots. Plate IX, fig. 2 shows a plant of Gola variety affected with leaf-drop streak and fig. 3 shows leaflets of the plant with veinal necroses and necrotic spots.

The symptoms of the disease in general in potato varieties Majestic, President, Windsor Castle and Talisman (Up-to-date) resemble those described for Gola.

The reactions of the cultures of the virus obtained from different potato varieties were studied on differential hosts and it was observed that the symptoms produced by different cultures on the differentials were almost similar.

The properties and reactions on differential hosts of the virus originally isolated in a pure form from a plant of Phulwa variety showing mosaic and veinal necroses are reported.

Reactions on differential hosts

A study of the symptom expression of the virus was carried out on a selected range of solanaceous plants including *Nicotiana tabacum* L., *Nicotiana glutinosa* L., *Nicotiana sylvestris* Spegaz and Comes and *Nicotiana rustica* L., *Datura stramonium* L., *Solanum nodiflorum* Jack., *Petunia hybrida* Vilm. and potato variety President. The reactions of the virus on these hosts are described.

Nicotiana tabacum (varieties Harrison's Special and White Burley). Primary symptoms of infection are visible about 15-17 days after inoculation in the form of clearing of the veins of young leaves. A couple of days later, vein clearing becomes very



FIG. 1. Leaflets of potato variety Phulwa showing veinal necroses
FIG. 2. Potato plant of variety Gola showing leaf drop streak
FIG. 3. Leaflets of the Gola plant showing veinal necrosis and necrotic spots



1



2



3



4

FIGS. 1 and 2. Leaves of *Nicotiana tabacum* showing vein clearing and green vein-banding
FIG. 3. Leaves of *Nicotiana glutinosa* showing green vein-banding
FIG. 4. Leaflets of Potato variety President showing veinal necroses

tingent. After a further period of about four days clearing tends to fade and is followed by a darkening of the green colour in the tissues along the veins. Green vein banding which is usually very prominent is a characteristic symptom of infection with this virus (Plate X, figs. 1 and 2).

Nicotiana glutinosa. Primary symptoms, i.e. clearing of the veins of young leaves, occur in about 15 days after inoculation. A week later vein-clearing appears and is replaced by green vein-banding. At times leaves also show some distortion (Plate X, fig. 3).

Nicotiana rustica and *N. Sylyestris*. Clearing of the veins of the young leaves commences about 17 days after inoculation. About 2-3 days later it becomes quite prominent and appears as chlorosis along the veins. Later green vein-banding occurs and is prominent on almost all the leaves.

Solanum nodiflorum. In about 15 days after inoculation leaves begin to show slight mottle along the veins. This later appears as distinct veinal mosaics characteristic of this virus.

Petunia hybrida. The first symptom of infection which appears about 16 days after inoculation is in the form of vein-clearing of youngest leaves. Darkening of the green colour along the veins is a subsequent development and is usually quite distinct. Leaves at times show some distortion.

Datura stramonium. Efforts to transmit the disease to this host by mechanical inoculation were unsuccessful. It was made sure by back inocula-

tions to *Nicotiana tabacum* that the disease was not carried.

Potato variety President. Infection of this host takes place about 12 days after inoculation when small necrotic spots appear on or near the veins. These later develop into streaks along the veins on the under surface of the leaf followed by distinct veinal necroses. The necroses then progress towards the petiole and finally result in leaf-drop streak (Plate X, fig. 4).

PROPERTIES OF THE VIRUS

Thermal inactivation. Exposure of the virus for 10 minutes in a water bath at various temperatures shows that the virus rapidly begins to lose activity after exposure to a temperature of 50°C. and at 54°C. the virus becomes innocuous.

Toleration to dilution. Inoculation of young tobacco plants with freshly extracted juice from diseased plant leaves as well as with juice diluted with sterilized distilled water showed that the infectivity begins to fall at a dilution of 1:100 and the virus is rendered innocuous at a dilution of 1:1000.

Longevity in vitro. Standard extract of the virus stored at laboratory temperature (29°-34.5°C.) was rendered innocuous after 24 hours whereas when the extract was stored at a lower temperature (8°-10°C.) it remained active for 32 hours and completely lost its infectivity after 36 hours.

The results of the experiments are summarized in Table I.

TABLE I
Properties of the virus

Thermal inactivation			Toleration to dilution			Longevity in vitro		
Exposure temperature °C.	No. of plants		Dilution	No. of plants		Storage period	No. of plants	
	Inoculated	Infected		Inoculated	Infected		Inoculated	Infected
Heated control	3	3	Nil (Control)	3	3	Fresh standard extract	4	4
"	3	3	1:10	3	3	" 4 hours	4	4
"	3	3	1:50	3	3	" 8	4	2
"	4	2	1:100	3	2	" 12	4	3
"	4	2	1:200	3	2	" 24	4	0
"	4	2	1:500	3	1	" 28	4	0
"	4	2	1:1000	3	0	" 32	4	0
"	4	1				" 36	4	0
"	4	0				" 48	4	0
"	4	0				" 72	4	0

Filtrability. Standard extract prepared from the young infected tobacco leaves was filtered through amberland filters of different grades (L₁-L₅) and it was found that the extract after passage through candles had been rendered inactive. The

filtration was carried out under reduced pressure of 1/5 atmosphere.

The reactions on differential hosts and the properties of the virus show that it is identical with Solanum virus 2 (Potato virus Y). The occurrence of this

virus in India was once reported by Pal [1943] as a result of the examination of diseased Phulwa plant tubers by Salaman in England. However, the occurrence of veinal necroses on Phulwa plants, an important diagnostic character of this virus on certain potato varieties, was not observed.

SUMMARY

The properties and reactions on differential hosts of a virus isolated from potato plant of Phulwa variety are reported.

The virus rapidly begins to lose activity after exposure to 50°C. and at 54°C. the virus becomes

innocuous. The virus is also rendered innocuous at a dilution of 1:1000 and after storage at room temperature for 24 hours. It is held back during passage through Chamberland filters (L_1 - L_5).

The virus is identical with *Solanum virus 2*.

ACKNOWLEDGEMENT

Thanks are due to Dr G. Watts Padwick, Imperial Mycologist, for his keen interest in the investigation

REFERENCE

Pal, B. P. (1943). Virus Diseases of Potatoes in India. *Curr. Sci.* **12**, 279

THE CYTOLOGY OF *CARICA PAPAYA* LINN.*

By L. S. S. KUMAR, A. ABRAHAM and V. K. SRINIVASAN, Botany Department, Agricultural College, Poona

(Received for publication on 4 September 1944)

(With Plate XI and 53 text-figures)

A THREE years' scheme financed by the Imperial Council of Agricultural Research for a preliminary investigation on the cytology of papaya, with a view to a subsequent study of sex inheritance, was started at the College of Agriculture, Poona, in July 1940. The main object of this scheme was to study the cytology of the different sex-types in papaya with a view to find out whether there are any visible chromosomal differences in morphology or behaviour during cell division, which could be correlated with sex expression. The present paper describes the findings of the above investigation.

THE GENUS *CARICA*

The botanical relationship of this genus has been the subject of much discussion and it has previously been classified under the families Passifloraceae, Cucurbitaceae, Bixaceae and Papayaceae, though it is now placed under Caricaceae along with the genus *Jacaratia*. The genus *Carica* comprises about 40 species all of which are indigenous to the American tropics, and are described as dioecious, except the cultivated species *C. papaya*, which is characterized by a variety of sex types possessing various flower types. Judging from the chromosome number of the seven species hitherto investigated, *C. chrysopetala*, *C. pentagona* and *C. candamarcensis* [Heilborn, 1921], *C. quercifolia* [Storey, 1941], *C. pubescens* [Kumar and Abraham, 1942], *C. dodecaphylla* [Kumar and Srinivasan, 1944], and *C. papaya* (reported in this paper and in previous studies), it

appears that the genus *Carica* is characterized by a uniform chromosome number of $2n=18$.

PREVIOUS WORK ON *CARICA PAPAYA*

The earliest contributions to our knowledge of the histology of *Carica papaya* were made by Usteri [1907] and Kratzer [1918] both of whom have studied the development of the embryo-sac. Usteri [1907] found that the micropylar cell of the tetrad gave rise to the embryo-sac, while Kratzer [1918] observed that any cell of the tetrad might function to give rise to the embryo-sac. In 1921, Heilborn made one of the first important contributions to the cytology and embryology of a few species of *Carica*. He examined cytologically four species of *Carica*, namely, *C. chrysopetala*, *C. pentagona*, *C. candamarcensis*, and *C. papaya*, in all of which he found the diploid number to be eighteen. Heilborn [1921] describes the development of the embryo-sac in *C. papaya* which is rather out of the normal; the megaspore mother cell divides, but no cell wall is formed and consequently no tetrad formation takes place. Both nuclei divide again to give four nuclei and of these four, one divides again. Thus an embryo-sac is produced with five nuclei, three of which form the egg apparatus, while the remaining two nuclei may be considered as polar nuclei, though no fusion of these two nuclei was observed. After fertilization, a non-cellular endosperm is developed and later the egg-cell divides and gives rise to the embryo. In *C. chrysopetala*, a parthenocarpic species lacking male flowers, Heilborn [1921] observed a bi-nucleate embryo-sac, each nucleus having nine chromosomes. He has also observed quite

* Findings of the investigation into the cytology of *Carica papaya* Linn., a scheme financed by the Imperial Council of Agricultural Research

large ovules with no archesporial cells, ovules with degenerating embryo-sacs, and many ovules with well-developed embryo-sacs. In *C. pentagona*, another parthenocarpic species lacking male flowers, normal reduction divisions were observed by Heilborn [1921] in some ovules. Reduction division in *C. candamarcensis* and *C. papaya* was found to be quite normal and in a single embryo-sac mother cell of *C. papaya* he had seen quite normal diakinesis. Meurman [1925] in a study of the chromosome behaviour of some dioecious plants with reference to the sex chromosomes has recorded the presence of nine gemini in the pollen mother cells of *Carica papaya* and the absence of any sex chromosome pair in them. Sugiura [1927] in a study of the meiosis of pollen mother cell of *C. papaya* confirms Meurman's [1925] observations besides reporting the formation of a multipolar spindle. Various investigations carried out by Sakurai [1929] to correlate sex with external characters of seedlings gave negative results. In 1930 Lindsay investigated *C. papaya* and came to the conclusion that no evidence could be found for an unequal pair of chromosomes in the division of the pollen mother cells. Asana and Sutaria [1929] in a study of the development of pollen in *C. papaya* found the meiotic chromosome number to be nine and also reported the occurrence of multipolar and tripolar spindles. Agharkar and Banerji [1930] have traced the development of embryo-sac in *C. papaya*. They found that the chalazal cell of a linear tetrad of megaspores gives rise to the mature 8-nucleate embryo-sac. Genetical studies in *C. papaya* of great importance were carried on independently by Hofmeyr [1938] at Nelspruit, S. Africa, and by Storey [1941] at Hawaii, on the inheritance of sex-forms and they came to identical conclusions. These studies have shown that (1) sex-determination in papaya could be explained in terms of simple Mendelian factors, (2) the female represents a homozygous recessive genotype, while the male and the hermaphrodites are enforced heterozygotes, (3) all combinations of homozygous dominant geno-types are lethal to the zygotes, (4) sex determination may be said to be of the XY type (i.e. male and bisexual XY; female XX), and (5) fertilization is entirely at random and there is neither differential selectivity nor differential viability among gametes. Linkage studies have shown that the sex factors for the different types occupy the same locus on homologous chromosomes along parts of which crossing over occurs freely. Their [Hofmeyr, 1938 and Storey, 1941] cytological observations led them to the conclusion that no heteromorphic pair of sex chromosomes is to be found in papaya. Storey [1941] in a bulletin gives a summary of all the work done up to 1941 on papaya in Hawaii, where investigations have been actively pursued for the past 30 years, and also his findings

on the cytology and genetics of *C. papaya*. The different sex types and flower types observed in *C. papaya* have been fully described in a recent publication [Kumar and Abraham, 1942].

MATERIAL AND METHOD

Papaya plants grown in India are usually from seeds of non-descript varieties, sometimes named after the locality of its origin, but in most cases of a mixed and unreliable type. On this account, as well as to make the study as comprehensive as possible it was felt necessary to obtain seeds from reliable sources in various countries and grow them in Poona for collecting material for cytological studies. Requests for seeds were accordingly sent to Agricultural Stations of most of the tropical and sub-tropical countries, where papaya is cultivated. The response to this was very good and more than 100 samples of seeds of different varieties were obtained. Fifty of these varieties selecting at least one sample from each country were raised in Poona. Some of these produced interesting sex forms and all material for this work was obtained from these plants. The object in growing such a large number of varieties was to find whether geographic isolation and effect of various climatic conditions have affected the chromosome make-up of the species, the original home of which is known to be South America, from where it has spread to the various tropical and sub-tropical countries in the world during the last three centuries.

Considerable difficulty was experienced in the beginning in obtaining good cytological preparations by using the common fixatives and staining methods. This was found to be mainly due to the presence of a large quantity of easily coagulable latex in the cells of even the floral parts. Attempts at dissolving the latex before or during fixation interfered with proper fixation. After trials with several chemicals, it was found that a modification of Bouin's fluid at a temperature of 35°C., applied after pre-treating the material for 2-3 minutes in Winge's Picro-carnoy, also at the same temperature, gave quite satisfactory results. Staining was mostly done by the iodine-gentian-violet method. But as this stain though unsurpassed for clarity and transparency of chromosome figures tends to fade rapidly after about six months, several new modifications of staining have been tried. One new method which gave good preparations is described below.

The slides are brought to water and then mordanted for about an hour in a mixture of equal parts of five per cent ferric ammonium sulphate and five per cent ferrous ammonium sulphate; then washed in running water for 10 minutes and stained in haematoxylin made according to Earle's [1939] formula, for about five minutes; then destained

in the first mordant diluted with equal part of water till the cytoplasm is clear and the chromosomes are stained only a light bluish black; next, the slides are washed in running water for about 15 minutes. After completing this abbreviated schedule of haematoxylin staining, the slides are stained with iodine-gentian-violet in the usual manner. This superimposing of gentian-violet stain on a light haematoxylin background gives a much better preparation than either of the two stains applied alone, particularly for prophase stages which are difficult to stain in plants in which chromosomes are very small. It has been noted that some preparations stained early in 1941 by this schedule still retain clearly the stain after three years.

For the detailed study of the morphology of somatic chromosomes, Lewitsky and others found that a fixative without acetic acid followed by the Feulgen staining was the best. This was particularly so in plants with small chromosomes. As in papaya the chromosomes are very small, it was thought that application of this method would reveal the details of morphology of the chromosomes more clearly than other methods. Repeated attempts at trying Lewitsky's methods failed to give satisfactory preparations and so the common fixatives containing acetic acid in various proportions had to be used. While this followed by gentian violet staining gave good results for study of number and gross morphology of chromosomes, the finer details were not always clear. Another method which gave good preparations may be mentioned here. Root-tips were fixed in Lewitsky's chrom-formalin mixture. The slides were taken down to 70 per cent alcohol and left overnight in a jar of 70 per cent alcohol to which a little lithium carbonate had been added. They were then brought down to water and stained with crystal violet for about two hours and then destained in the usual way.

Anthers of correct stages were smeared and fixed in Belling's Navashin for two hours, after which they were washed, taken up to 70 per cent alcohol and left overnight in 70 per cent alcohol with a little lithium carbonate. They were then stained in crystal violet as described above. This method gave particularly good preparations in which large number of p.m.c.s with Metaphase I plates were obtained. Both side views and polar views of first metaphase were obtained. In the side view of Metaphase I plates, the chromosome complement of a number of pollen mother cells could be clearly analysed and the polar view of M-I plates exhibited very clearly various types of secondary association. These methods were supplemented with aceto-carmine smears, which facilitated examination of a large number of plants.

For the study of megasporogenesis, slices of ovaries of different stages of development were fixed either

in hot corrosive sublimate, formalin-acetic-alcohol or in Zenker's fluid after a minute's prefixation in Winge's picro-carnoy. They were sectioned at thicknesses varying from 8 μ to 14 μ and stained in Haidenhein's iron-alum haematoxylin.

In Table I is given a list of the different varieties of papaya that have been examined cytologically and their source, and also the chromosome numbers of two other species of *Carica* namely *pubescens* and *dodecaphylla*.

OBSERVATIONS

The meiosis in the pollen mother cells of twenty-one varieties of papaya have been examined by permanent preparations. Meiosis of three of these (variety Nos. 15, 46 and 99) have been studied in the hermaphrodite also; while in one—variety No. 46—meiosis has been studied in detail in all the three sex types, viz. male, female and hermaphrodite. In addition to these, a few more varieties have been examined both in the male and the bisexual through temporary aceto-carmine smears. Analysis of the chromosomes of these sex-types has shown that the haploid number is $n=9$ for all of them. Slight morphological differences among the bivalents have been observed. The varieties also showed slight variations in the size of the chromosomes. Apart from these minor variations, meiosis in both male and hermaphrodite was found to be normal. Comparison of the chromosomes of the male and the hermaphrodite showed no differences which could be correlated with the differences in sex-expression. Somatic chromosomes of twelve varieties have been examined and the number found to be $2n=18$. Here also distinct morphological differences in the chromosomes have not been observed. All the chromosomes have median or sub-median constrictions. The length of somatic chromosomes at metaphase varies from 2 to 3 μ , while the length of meiotic chromosomes at metaphase in the pollen mother cells (as determined from enlarged photomicrographs) is about one-tenth of the length at somatic metaphase.

One variety of *Carica papaya* (variety No. 98), seeds of which were got from Chengannur in Travancore State, is the most distinct in respect of its vegetative characters. It is characterised by the presence of yellow colour in all parts of the plant. Fruits are bright yellow from the early stages. Only the leaf blades have a greenish colour mixed with yellow. There are no male plants in this variety. Examination of the pollen mother cell in the hermaphrodite showed that the chromosomes number in this variety also is $n=9$. Reference to literature (which is by no means comprehensive) shows that this is an undescribed variety.

Mitosis. It has been observed that when seeds from fruits of female plants growing in close proxi-

TABLE I

A list of different varieties of papaya examined

No.	Varietal name	Locality	Source	Chromosome No.	
				<i>n</i>	<i>2n</i>
1	Madhubindu . . .	Kathiawar . . .	Ramji Hansraj & Sons, Kathiawar . . .	9	18
2	Alipore . . .	Alipore . . .	Secretary, Royal Agri-Horticultural Society, Calcutta . . .	9	18
3	Honeydew . . .	Baroda . . .	Horticulturist to Government, Baroda State . . .	9	18
4	Mixed-sweet . . .	Coimbatore . . .	Superintendent, Central Farm, Coimbatore . . .	9	18
5	Kodur 3/1 . . .	Kodur . . .	Sri K. C. Naik, Kodur Farm . . .	9	18
6	Kodur 3/2 . . .	Kodur . . .	Ditto . . .	9	18
8	Kodur 1/4 . . .	Kodur . . .	Ditto . . .	9	18
9	Kodur 13/4 . . .	Kodur . . .	Ditto . . .	9	18
12	Singapore . . .	Malaya . . .	Central Experimental Station, Serdang, Selangore, F.M.S. . . .	9	18
15	Washington . . .	Poona . . .	Horticulturist to Government, Bombay, Poona . . .	9	18
29	Solo . . .	Netherlands . . .		9	
34	<i>Carica hybrida mexicana</i> . . .	S. America . . .			18
40	Genetic cross . . .	S. Africa . . .	J. D. J. Hofmeyr, Nelspruit, S. Africa. . .	9	
44	Kuching . . .	Saravak . . .		9	
46	Formosa . . .	Formosa . . .	Dr Matsuura, Hokkoide, Imperial University, Sapporo, Japan . . .	9	18
47	Heito-Delicious . . .	Japan . . .	Ditto . . .	9	
54	Kuching . . .	Hawaii . . .	Dr W. B. Storey, Hawaii . . .	9	
55	Kuching . . .	Hawaii . . .	Ditto . . .	9	
56	Marumi ogata . . .	Japan . . .	Dr H. Matsuura, Japan . . .	9	
98	Yellow papaya . . .	Travancore . . .	Mr A. T. Abraham, Chengannur, Travancore State . . .	9	
99	Solo papaya . . .	Australia . . .	Dept. of Agriculture, Queensland. . .	9	
105	Florida green . . .	British Guiana . . .	Director of Agriculture, Guiana . . .	9	
	<i>Carica pubescens</i> . . .	Cuba . . .	Secretary for Agriculture, Cuba, and British Consulate General, Buenos Aires . . .		18
	<i>Carica dodecaphylla</i> . . .	Argentina . . .			18

mity to male plants are sown, the resulting progeny shows an approximately equal proportion of female and male plants with a few hermaphrodites. So a study of the somatic chromosomes in the root-tips of a number of young seedlings should show whether there is any difference between the chromosome make-up of the male and the female. With this object in view, seeds from a single fruit (of variety No. 34) obtained by open pollination were sown and the tips of the main root in about 100 seedlings were fixed. Examination of the metaphase chromosomes from a number of sections showed that the differences from root-tip to root-tip were not greater than those observed from cell to cell in the same section. Figs. 16 to 18 show somatic metaphases from different root-tips. Unless there is a marked difference between chromosomes of one sex and another, a study of this type may not be expected to reveal any differences that may exist. This is particularly so in a case like papaya where the chromosomes are extremely small in size. However, this study shows that no marked differences exist between the chromosomes of the male and the female in the same

variety. Further, from independent genetical studies Hofmeyr [1938] and Storey [1941] came to the identical conclusion that in papaya male and hermaphrodite are heterogamous for sex, while the female is homogamous. So, if at all a heteromorphic pair of chromosomes carrying the genetic factors for sex is present, it should be expected in the male or hermaphrodite and not in the female.

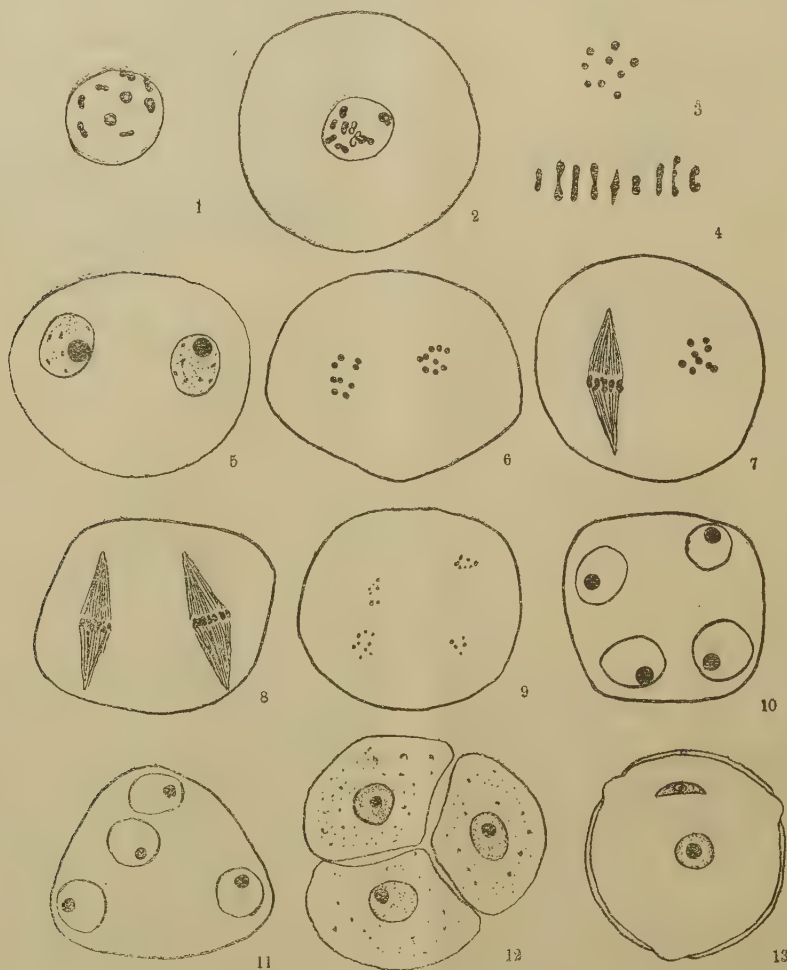
As previously stated, variety No. 46 was selected for a critical and comparative study of meiosis in male, female and hermaphrodite sex-types, with a view to settle once for all the question whether there are any visible chromosomal differences in morphology or behaviour during cell division which could be correlated with sex expression.

Meiosis in hermaphrodite of variety 46. In the hermaphrodite sex-type there are only five stamens, while in the male, there are ten stamens.

In very young anthers, the pollen mother cells remain packed together closely and are in close contact with the tapetal cells which form a ring around them. They begin to round off at about the stage of diakinesis. In aceto-carmin smears

also, this is the earliest stage at which clear visibility of chromosomes is possible. The diakinetik stage is preceded by a 'diffuse stage' when the chromosomes are not stained properly. This observation was made from a large number of permanent preparations, in which the prophase and stages after diakinesis are well-stained, whereas the stage immediately preceding diakinesis is not stained. Such a feature has been noted in other plants also.

At late-diakinesis, the eighteen chromosomes which form nine pairs are distributed along the periphery of the nucleus (Fig. 1). The nucleolus has disappeared at this stage. The bivalents themselves may be either of the rod-type or of the ring-type. After examination of a large number of diakinetik stages, it was found that of the nine bivalents, one to three are of the ring-type, while the rest are all rod-shaped.



FIGS. 1-13—*Carica papaya*—Hermaphrodite plant of variety No. 46

- FIG. 1. Diakinesis in pollen mother cell. 3 bivalents are ring-shaped while the remaining 6 are of the rod-type. $\times 1667$.
 FIG. 2. Prometaphase of meiosis. $\times 1667$.
 FIG. 3. Polar view of metaphase I. $\times 1667$.
 FIG. 4. Lateral view of metaphase I. $\times 1667$.
 FIG. 5. Interkinesis nuclei. $\times 1167$.
 FIG. 6. Metaphase II. Both the metaphase plates in polar view. $\times 1167$.
 FIG. 7. Metaphase II. One of the metaphase plates in

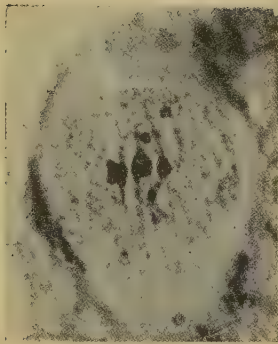
- polar view while the other shows the side view. $\times 1167$.
 FIG. 8. Metaphase II. Both the metaphase plates in side view. $\times 1167$.
 FIG. 9. Telephase of second division. $\times 1167$.
 FIG. 10. Iso-bilateral type of pollen tetrad formation. $\times 1167$.
 FIG. 11. Tetrahedral type of pollen tetrads. $\times 1167$.
 FIG. 12. The young microspores. $\times 1167$.
 FIG. 13. Two-celled microspore at the time of shedding. $\times 800$.



1.



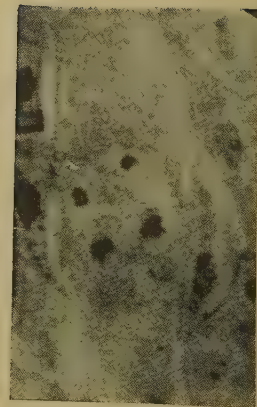
2.



3



4



5

FIGS. 1—4. *Carica papaya*—male plant of variety No. 46

FIG. 1—Metaphase I exhibiting secondary association : 3(2)—3(1). $\times 3000$

FIG. 2—Metaphase I exhibiting secondary association : 1(3)—2(2)—2(1). $\times 3000$

FIG. 3—Precocious separation of one bivalent. $\times 2500$

FIG. 4—Nuclear division in the generative cell of the pollen tube. $\times 2500$

FIG. 5—*C. papaya*—female of Var. No. 46. Diakinesis in m.m.c., four out of the nine bivalents are in focus. $\times 2500$

During the next stage of prometaphase (Fig. 2) bivalents undergo a converging movement, and when they are in close assemblage in the centre of the nucleus. Metaphase follows prometaphase. The nine bivalents are arranged on the equatorial plate (Fig. 3). A characteristic feature of metaphase is the association or approximation of the different bivalents. This secondary association which is the pairing of ancestrally related bivalents has been studied in detail in the male of this variety. The evidence is available to support the findings of earlier investigators that metaphase is a very transient stage, as numerous cells with the bivalents arranged in one plane are not met with. But in some cases such arrangement in one plane has been met with and in these secondary association is unmistakably evident. The size of the bivalents varies from 0.2 to 0.3 μ . Fig. 4 shows the side view of the nine bivalents at metaphase I. The separation of the bivalents during anaphase is regular and regular, though very occasional lagging of spindle has been observed. At the end of the first metaphase, two interkinesis nuclei are formed (Fig. 5). Sugiyama [1927], Lindsay [1930] and Asana and Sutaria [1930] have reported the presence of multi-axial spindles. Occasionally it has been met with in the present study also.

The second division is quite normal and nine chromosomes have been counted in both metaphase plates in the same pollen mother cell (Fig. 6). The chromosomes are smaller than in the first division. No difference in size apparent in the first division is not seen at the second. Fig. 7 shows a second metaphase plate, where one of the groups is in the polar view while in the other the side view is seen. Secondary association persists in second metaphase also. The further meiotic process (Figs.

8 and 9) is regular resulting in tetrads, which may be either tetrahedral (Fig. 11) or isobilateral (Fig. 10). Wall formation is by furrowing (Fig. 12) as already reported by Asana and Sutaria [1928]. The mature pollen grain at the time of shedding is bi-nucleate (Fig. 13).

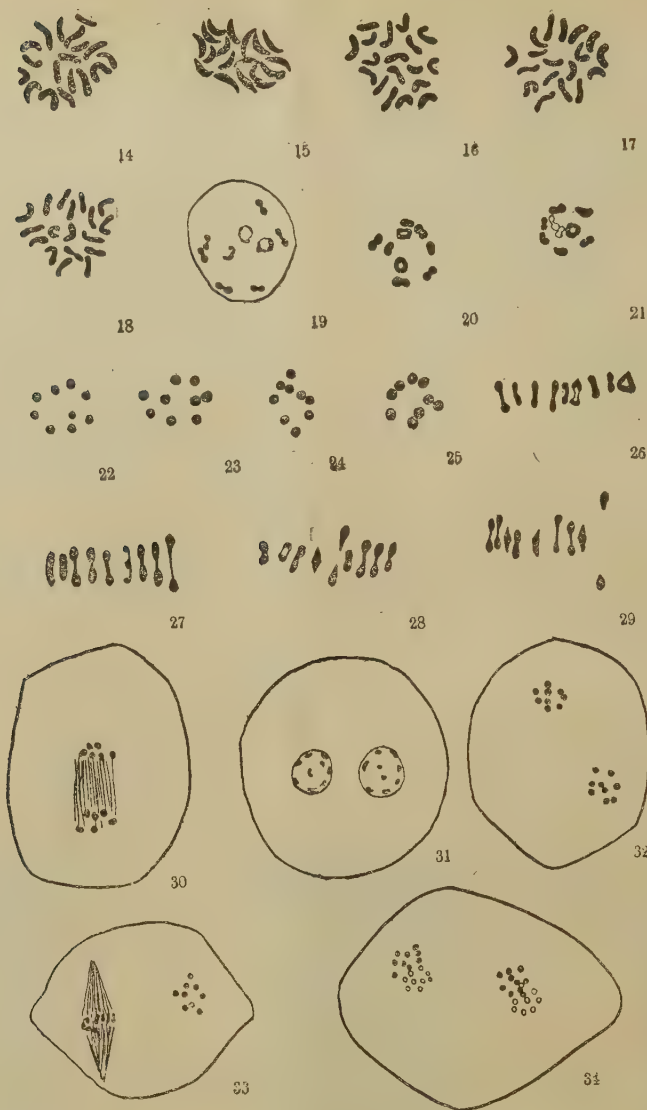
Meiosis in male of variety 46. At early diakinesis nine bivalents are observed distributed peripherally along the nucleus (Fig. 19). The nucleolus is seen very faintly stained. Fig. 20 shows late diakinesis. Two of the bivalents are of the ring-type, while the remaining seven are rod-shaped. At diakinesis, the two chromosomes of a bivalent could be clearly seen and careful comparison of the size and shape of the two members of each pair revealed no inequality between the chromosomes of any of the pairs. During pro-metaphase (Fig. 21) bivalents are in close assemblage in the centre of the nucleus. At metaphase the bivalents again are separated and arrange themselves on the equatorial plate (Fig. 22). The characteristic feature of metaphase is the association or approximation of the different bivalents. This secondary association is prevalent in the majority of pollen mother cells. Out of 62 first metaphase plates examined, 44 exhibit secondary association, while the rest do not. The paired bivalents are similar in size and configuration. A variable number of bivalents are seen to be secondarily associated. Fig. 22 shows the stable configuration, which consists of one bivalent in the centre with the remaining eight arranged in a ring around it. Figs. 23 to 25 and photomicrographs (Plate XI, figs. 1 and 2) show various degrees of secondary association. This pairing is exhibited very clearly at the first and second metaphase.

Table II gives a summary of the various types of secondary association.

TABLE II

A summary of various types of secondary association

No. of associations	No. of bivalents in association				No. of cases	Total
	1	2	3	4		
1	7	1	..	..	24	24
2	{ 5 6	2 ..	.. 1		{ 11 3	14
3	{ 3 4 5	3 1 ..	.. 1 ..	 1	{ 3 1 1	5
4	1	4	..	..	1	1
						Total 44



Figs. 14-34

FIG. 14. Metaphase plate from root-tip of *C. dodecaphylla*.
 $2n=18$. $\times 2167$.

FIG. 15. Metaphase plate from root-tip of *C. pubescens*.
 $2n=18$. $\times 2167$.

Figs. 16-22.—*Carica papaya* (male plant)

FIGS. 16-18. Metaphase plates from root-tips of different seedlings of *C. papaya* (Variety No. 34). $2n=18$. $\times 2167$.

FIG. 19. Early diakinesis. Two of the bivalents are ring-shaped. $\times 1667$.

FIG. 20. Late diakinesis. $\times 1667$.

FIG. 21. Prometaphase. $\times 1667$.

FIG. 22. Metaphase I. One bivalent is in the centre with eight others arranged around it. $\times 1667$.

FIGS. 23-25. M-I plates showing different degrees of secondary associations. Fig. 23: 1(2)-7(1); Fig. 24:

2(2)-5(1); Fig. 25: 4(2)-1(1); (maximum association). All. $\times 1667$.

FIG. 26. Side view of M-I. $\times 1667$.

FIG. 27. Same as above. Note the bivalent on the extreme right about to segregate. $\times 1667$.

FIG. 28. One bivalent separating precociously. $\times 1667$.

FIG. 29. Same as above showing a later stage. The segregating chromosomes are similar. $\times 1667$.

FIG. 30. Anaphase I. $\times 1167$.

FIG. 31. Interkinesis nuclei. $\times 1167$.

FIG. 32. Metaphase II. Polar view. Two groups of nine chromosomes each are seen. $\times 1167$.

FIG. 33. Same as above. One of the plates in side view. $\times 1167$.

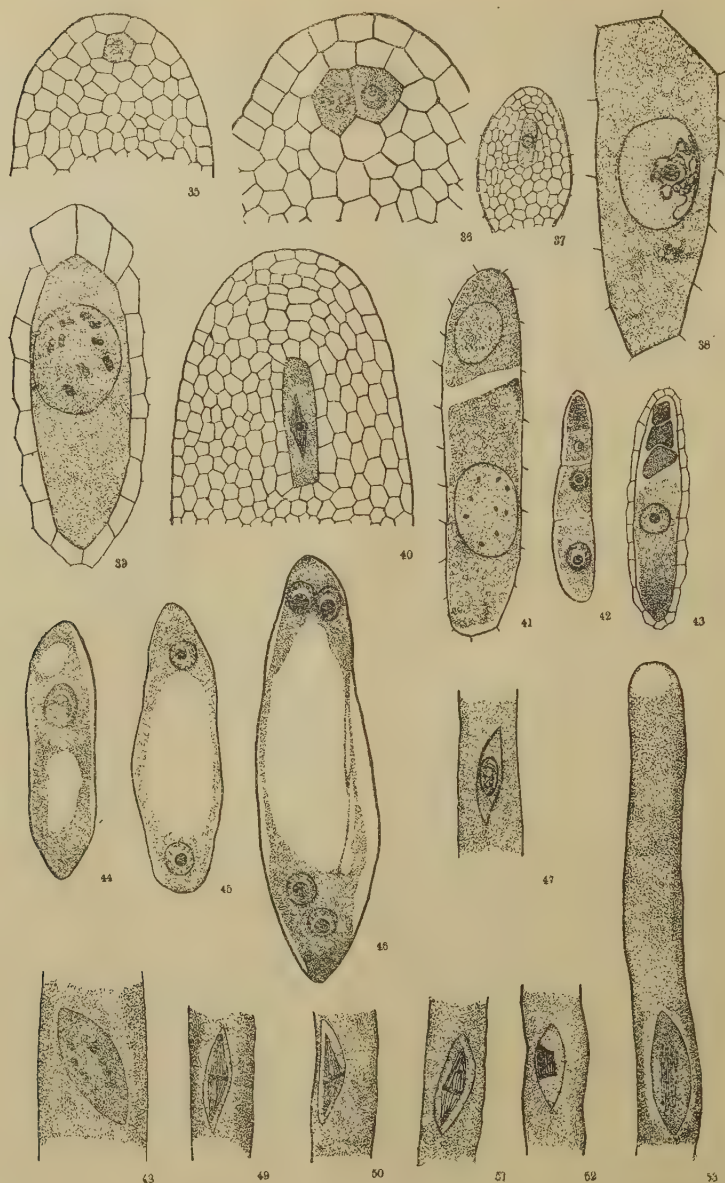
FIG. 34. Anaphase II. Four groups of nine chromosomes each are seen.

In computing the number of secondary associations in each case, an association of four bivalents to one group is counted as three, that of three bivalents as two, and that of two bivalents as one. The number of secondary association per plate ranges from one to four, the mode being one which occurs in 24 out of 44 cases. The maximum of four associations was seen once in 44 plates examined. Plate XI shows four groups of two bivalents each and a single bivalent, on the whole making five separate associations (Fig. 25). Subsequently, another case of maximum of four associations was met with, which showed one group of three bivalents, two groups of two bivalents each, and two unassociated bivalents (Plate XI, fig. 2).

A large number of metaphase plates in lateral view were examined. The outline of all the nine bivalents is clearly distinguishable in side view and Figs. 26 and 27 represent two such metaphase plates. The bivalents have been separated laterally while drawing. This fact namely that all the nine chromosome pairs are distinguishable on a number of slides in lateral view, makes possible the definite conclusion that in papaya there are no morphological differences recognizable by methods at present available between the two chromosomes of any pair in a pollen mother cell. In very early anaphase stages, the spindle fibres have already arranged themselves into a bipolar spindle, and the two members of each bivalent begin to be drawn apart. In such stages where the chromosomes of the different pairs are just beginning to separate, it was often found that in one particular bivalent, the paired chromosomes have already separated and are well on their way to the poles (Figs. 28 and 29). Plate XI, fig. 3 shows clearly one such plate, where, while the eight bivalents are still on the equatorial plate, one bivalent, however, has separated, the chromosomes of which are about half way through on their way to the poles. This interesting phenomenon was observed in about 10 cases out of about 15 of exactly the same stage examined. This precocious anaphasic separation of one of the bivalents is interesting, because, such a precocious separation during early anaphase is a characteristic feature of the sex-chromosome pair. This has been found by Moto [1928] to be a common feature of the sex chromosomes of the dioecious species in both dicotyledons and monocotyledons that he had studied. This phenomenon has again been recorded in *Coccinia indica* by Kumar and Deodikar [1940]. After a critical examination of the chromosomes that separate precociously in all the 10 cases reported, it can be said that there are no visible morphological differences between the chromosomes of the bivalent that exhibit precocious separation. In second metaphase plates, two groups of nine bivalents each could be clearly seen (Fig. 32).

Secondary association persists in anaphase I and metaphase II. The subsequent stages in meiosis leading up to the formation of tetrads are quite normal and similar to that described already in the case of the hermaphrodite plant.

Microgametogenesis. Pollen grains from the male plant of variety No. 46 were germinated on an agar-sugar culture medium and the nuclear division of the generative cell in the pollen tube was studied. The method employed is the one described by Maheshwari and Wulff [1937]. The mature microspore at the time of shedding is spherical, two-celled and possesses three germ pores (Fig. 13). After preliminary trials, it was found that germination of the pollen grains was best in a culture medium consisting of agar and sugar in the ratio of 1 : 10. For getting the requisite growth in length of the pollen tube, after which the generative cell begins to divide, it was found necessary to allow the microspores to germinate in the culture medium for about twenty hours. The slides were fixed in Formalin-Acetic-Alcohol for about three hours and stained in iron alum haematoxylin. The pollen tube begins to grow from one of the germ pores and as it goes on elongating, the crescent shaped generative cell moves down into the pollen tube and is about 6μ distant from the free end of the pollen tube, when it begins to divide (Fig. 53). The pollen tubes showing division stages are about 40μ in length. The tubes are comparatively broad, the breadth being about 1μ . In Fig. 47 is represented a pollen tube with the nucleus of the generative cell in the prophase of division. Figs. 49 and 50 show lateral views of the metaphase stage. The generative cell usually lies in the middle of the pollen tube though occasionally it may be close to one wall (Fig. 50). Plate XI, fig. 4 is a photomicrograph of a similar stage. The cytoplasm of the generative cell is differentially stained from that of the tube and the outline of the generative cell stands out markedly from the cytoplasm of the tube. Fig. 48 shows a polar view of the metaphase. Nine chromosomes are seen. No differences are noticeable between the chromosomes. The frequent arrangement of chromosomes in a line, one below the other, is not met with in the present case, as apparently this type of spatial distribution of chromosomes is necessary only when the chromosomes are relatively large as in *Lilium* and other genera [Q'Mara, 1933]. Since in the present case, the tube is broad and the chromosomes are rather small, there is no necessity for such a linear arrangement of the metaphase chromosomes and so they arrange themselves in a plate in the normal way. In Fig. 51 is seen what appears to be an unseparated chromosome lying outside the plate. Anaphase is normal and is shown in Figs. 52 and 53. The later stage showing the organized male gametes was not observed.



FIGS. 35-53

- Figs. 35 to 46 of *C. papaya* (female plant)
- FIG. 35. Single hypodermal archesporial cell. $\times 380$.
- FIG. 36. A plate of two archesporial cells with a layer of wall cells. $\times 600$.
- FIG. 37. Megaspore mother cell with about five layers of parietal tissue. $\times 250$.
- FIG. 38. M.M.C. with the nucleus in the prophase stage. $\times 1250$.
- FIG. 39. M.M.C. with the nucleus in diakinesis of the heterotypic division; nine bivalents can be counted. $\times 1250$.
- FIG. 40. M.M.C. deeply embedded in the nucellus (side view) of the heterotypic division. $\times 550$.
- FIG. 41. Dyad with the two nuclei about to divide again. $\times 1250$.
- FIG. 42. A linear tetrad of megaspores. $\times 600$.
- FIG. 43. Same as above, showing the functional chalazal megaspore and the three degenerating megaspores. $\times 600$.

- FIGS. 44-46. One, two and four nucleate embryo-sacs respectively. $\times 1250$.

- Figs. 47 to 53 of *C. papaya* (male plant)
- FIG. 47. Spindle-shaped generative cell in pollen tube, with its nucleus in the prophase stage. $\times 875$.
- FIG. 48. Metaphase stage in the division of the generative nucleus. Nine chromosomes are seen. $\times 1250$.

- (Figs. 49 to 53 magnified 875 times)
- FIG. 49. Lateral view of metaphase stage. The generative cell lies in the middle of the pollen tube.
- FIG. 50. Same as above. The generative cell lies close to one wall of the pollen tube.
- FIG. 51. Same stage as above. One of the chromosomes lying outside the plate.
- FIG. 52. Anaphase of the division of generative nucleus.
- FIG. 53. Same as above showing the distance of the generative cell from the tip of the pollen tube.

Meiosis in female of variety 46. In *Carica papaya* there are numerous anatropous ovules that arise not only from the placental region, but sometimes also from the entire inner wall of the ovary. All the ovules do not develop to maturity. A good number of the ovules degenerate in the early stages of their development. In very young ovules, a single primary archesporium is differentiated as a hypodermal cell, which is larger and takes a deeper stain than the other cells of the ovule (Fig. 35). In a solitary case, however, a plate of two archesporial cells with the primary wall cells already cut off was noticed (Fig. 36), though no case of two megaspore mother cells was observed in the later stages in the development of the archesporial cells into embryo-sacs. The primary archesporium cuts off a parietal or wall cell and functions directly as the megaspore cell. As the ovule develops, this wall cell by repeated periclinal divisions forms a parietal tissue of about 6 to 8 cells in thickness. As a result of the formation of this thick parietal tissue the megaspore mother cell is pushed deep into the nucellus. By this time, the megaspore mother cell has increased in size considerably. Just when the archesporial cell has cut off the parietal cell, the inner of the two integuments arises from the side of the ovule. The initiation of the outer integument follows in close sequence and it grows more rapidly than the inner integument which is completely enveloped by the outer integument when fully developed. The integuments are 3-4 cells thick. Both the integuments take part in the formation of the micropyle. Fig. 38 shows a fully enlarged megaspore mother cell with its nucleus in the prophase stage. In Fig. 39 is observed a megaspore mother cell nucleus in the diakinesis stage of the hetero-typic division. Nine bivalents are clearly observed at this stage. The bivalents are distributed peripherally in the nucleus and are therefore in different foci. About 10 such stages of the megaspore mother cell nucleus in diakinesis were observed. A careful examination of all of them showed no difference between the chromosomes of any of the pairs. Their behaviour during meiosis also seems to be quite normal. Preparations showing side views of the meiotic metaphase were available (Fig. 40). In such side views, all the nine bivalents could not be identified though the profile of about 5 or 6 of the bivalents could be made out clearly. Anaphasic separation is quite normal and the two daughter cells of the resulting dyad (Fig. 41) again divide to give rise to a linear tetrad of megaspores (Fig. 42). Invariably, in all cases, it was the chalazal megaspore that was functional while the rest degenerated (Fig. 43). The functioning megaspore which is full of cytoplasm enlarges in size considerably to form the uninucleate embryo-sac (Fig. 44). The single nucleus divides. The two

daughter nuclei move towards the poles of the embryo-sac and a central vacuole is formed (Fig. 45). Another division of these two nuclei gives rise to a four-nucleate embryo-sac (Fig. 46). Further stages in the development of the mature embryo-sac were not studied. The development of the embryo-sac so far described is in agreement with that already found by Agharkar and Banerji [1930].

DISCUSSION

Secondary association. The occurrence of marked secondary association of the bivalents in papaya has been recorded for the first time in the present investigation. About 70 per cent of metaphase I plates exhibit secondary association. From Table II, it is evident that the most frequent association is into eight groups (one group of two bivalents and seven separate bivalents), while the maximum association observed in a solitary case is into five groups (four groups of two bivalents each and one single bivalent). The criterion for deducing the basic chromosome number of a genus from secondary association, is the maximum association observed. In the present case, the maximum association is into five groups, and as such the basic chromosome number would be five for the genus *Carica*. In this connection, a detailed study of the other species of *Carica* may throw more light on the question of the basic chromosome number of this genus.

The cytological basis of sex expression. The discovery of a morphologically distinguishable difference between the chromosome complements of the male and female in animals, where generally the two sexes are found on separate individuals, was followed by the finding that sex-linked genes are located on these extra or accessory chromosomes, which came to be known as *sex-chromosomes* as distinguished from the autosomes, which are identical in both sexes. A similar cytological situation was found in a number of dioecious flowering plants also. In a large number of investigated cases including many species of insects, most of the mammals, and in several dioecious plants, the females have two X chromosomes, the male but one. In most of these, the males have another chromosome, the Y chromosome, but in some instances, this is absent. Thus the female is XX or homogametic, while the male is XO or XY and heterogametic. While this is the common condition, variations from this are met with in a number of cases. The occurrence of 'intersexes', in the moth *Lymantria dispar*, led Goldschmidt [1934] to propose a modification of the simple explanation of sex determination described above. According to his 'balance theory of sex', sex development (male or female) is the result of a competition between opposed tendencies in which the final expression is determined by the relative strengths or balance existing

CRYPTOSTEGIA GRANDIFLORA R.Br., A WAR TIME SOURCE OF VEGETABLE RUBBER

V. METABOLIC STUDIES*

By RAFAT HUSAIN SIDDIQUI, M.Sc., Ph.D., D.Phil. (Oxon), Organic Chemist and S. A. WARISI, B.Sc. (Hons.) (ALLD.), Phipps Chemical Laboratories, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 28 February 1945)

(With Plate XII and one text-figure)

THE object of the investigation was to study metabolic changes of the seedlings of *Cryptostegia grandiflora* with relation to their growth. The seeds after soaking in water were sown in the nursery and daily observations were made on 10 seedlings. The percentages of nitrogen, inorganic constituents and resins have been given but the estimation of rubber could not be carried out for reasons given in the experimental section and would be taken up again.

EXPERIMENTAL

The seeds (1 oz. comprising about 3,000) of *Cryptostegia grandiflora* were soaked in water for 24 hours after presoaked seeds give on sowing a quicker and more uniform germination than dry seeds.

The soil of the nursery after manuring it with farmyard manure (eight cart loads per acre) was worked to a fine tilth and levelled by means of a levelling board. On 23 September 1943, the presoaked seeds were evenly spread over a flat bed (21 sq. ft.) with the hand. They were then lightly covered with soil by passing a hand rake over the bed, and were about $\frac{1}{4}$ - $\frac{1}{2}$ in. deep in it. The surface of the bed was then gently evened out and the soil on the top was compacted by pressing by a light wooden roller. The nursery was then hand-watered and covered with gunny sheets (a mulch of *bhusa* about $\frac{1}{2}$ in. thick would also serve the purpose). Thereafter it was hand-watered twice daily, morning and evening. The gunny mulch helped to conserve the moisture in the soil and produced a humid atmosphere necessary for the proper germination of the seeds. When germination started on 27 September 1943, gunny sheets were removed. In about a couple of days over 90 per cent of the seeds had germinated, the stand of the seedlings being quite uniform.

On the fourth day of sowing the seedlings showed differentiation into yellow cotyledons, white plemule

and roots. After one week the seedlings showed pink stems and green leaves. In the first fortnight there was only one pair of leaves, in the third week there were two and three pairs of leaves and two months old seedlings had five and six pairs of green leaves (Plate XII, fig. 1). The stems remained pink. For the analysis 10 seedlings were carefully dug out intact, washed with water from the adherent soil and the fresh weight was taken after drying them between folds of filter paper. The seedlings were kept over sulphuric acid for about two months when they were dried up completely and turned brittle. The dry seedlings were crushed and kept in a packet of filter paper and several of these packets were extracted at a time with alcohol to find out the amount of solubles or resins. The material of every packet was then dried at 90°C. in an air oven and from the loss it suffered during extraction the percentage of resins was calculated. The resin-free material was re-soxhleted with benzene for estimating its rubber content, but it showed no loss in weight and was further extracted with chloroform in an apparatus designed by the authors. The apparatus consisted of a cone made of wire gauze and was placed in the distillation apparatus on a triangular stand made of wire. This cone was fitted with a cone of filter paper in which several of the packets containing the material were kept for extraction (Plate XII, fig. 2).

After extraction for 36 hours the material of every packet was dried at 90°C. It did not suffer any loss in weight but, on the contrary, showed a slight increase. The rubber could not thus be estimated in the seedlings. The very fact that benzene and chloroform failed to extract rubber from the seedlings dried over sulphuric acid for two months was indicative of a change that rubber underwent; the latter became converted into such substances which are insoluble in rubber solvents. Inorganic constituents (ash) and total nitrogen of the seedlings were also determined but the quantity of the first 15 samples was not enough for estimating both and so only their nitrogen (by Kjeldahl method) was determined. The growth observations as well

* Parts I and II of this series were published in the *J. Indian Chem. Soc.* (1944) and parts III and IV in the *J. Indian Chem. Soc. Ind. and News Ed.* (1945).

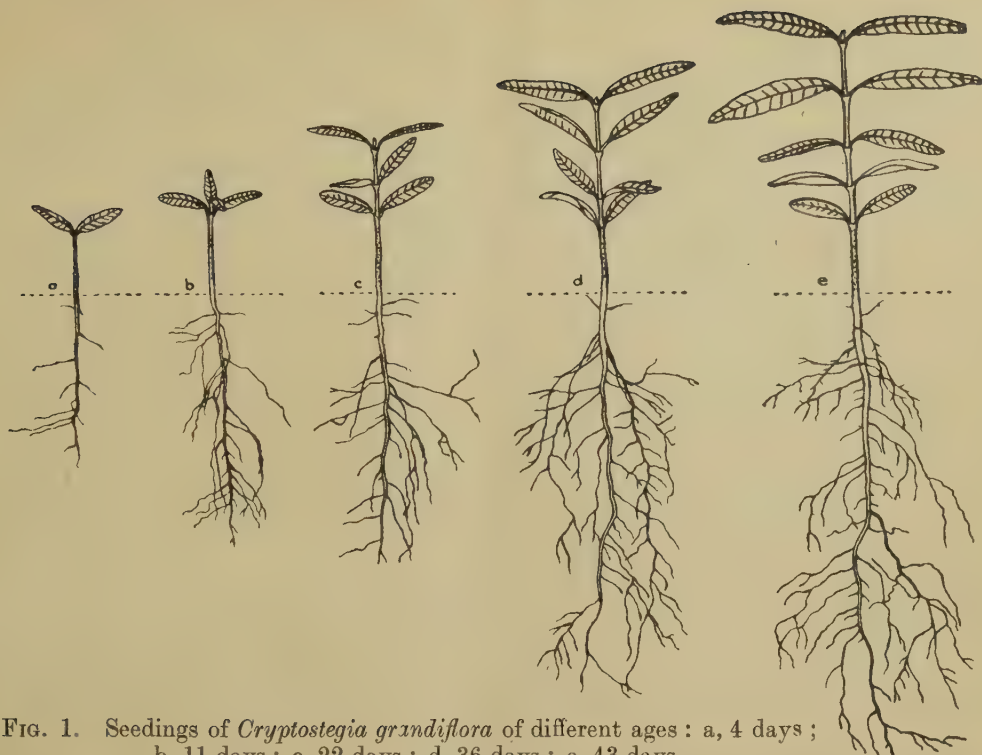


FIG. 1. Seedlings of *Cryptostegia grandiflora* of different ages : a, 4 days ; b, 11 days ; c, 22 days ; d, 36 days ; e, 43 days

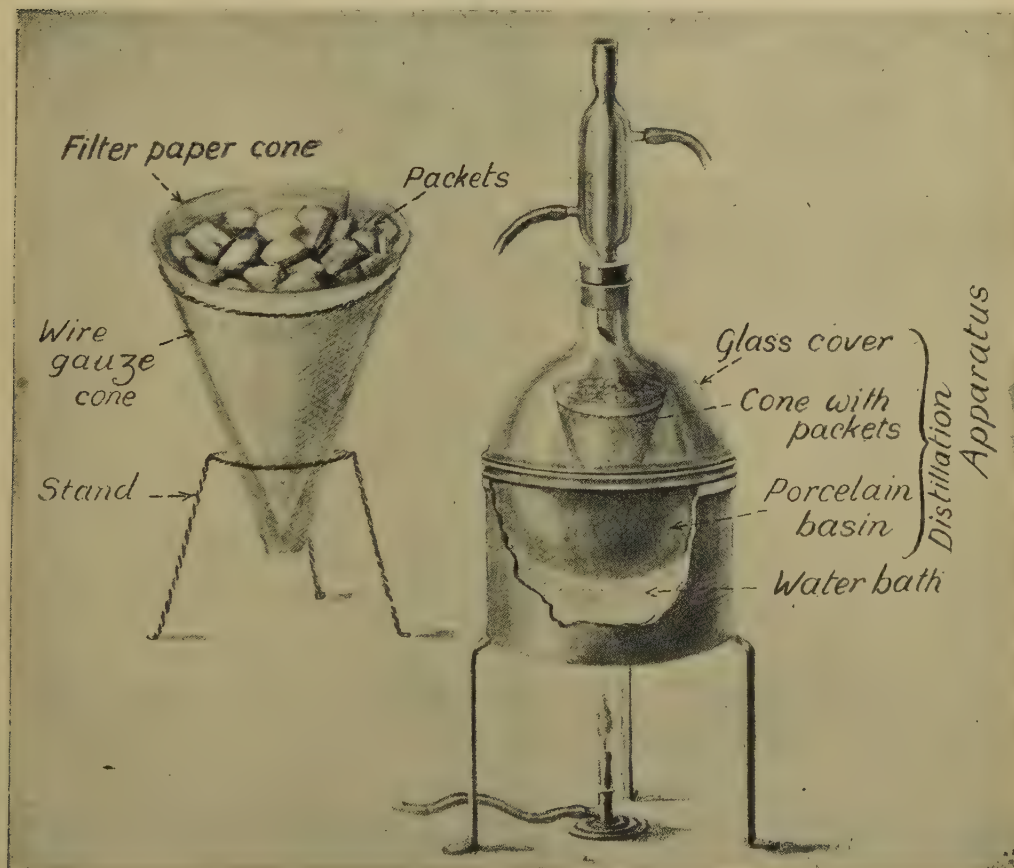


FIG. 2. Apparatus for extraction with chloroform

the results of the analyses have been given in Table I. The results show that as growth proceeds and the polymerisation of the products of the photosynthetic activity results in the formation of cellulose,

etc. there is a gradual decrease in the percentage of inorganic constituents (16-10 per cent) and nitrogen (6-3 per cent) while resins show a fluctuation within a certain range (46—26 per cent) (Fig. 1).

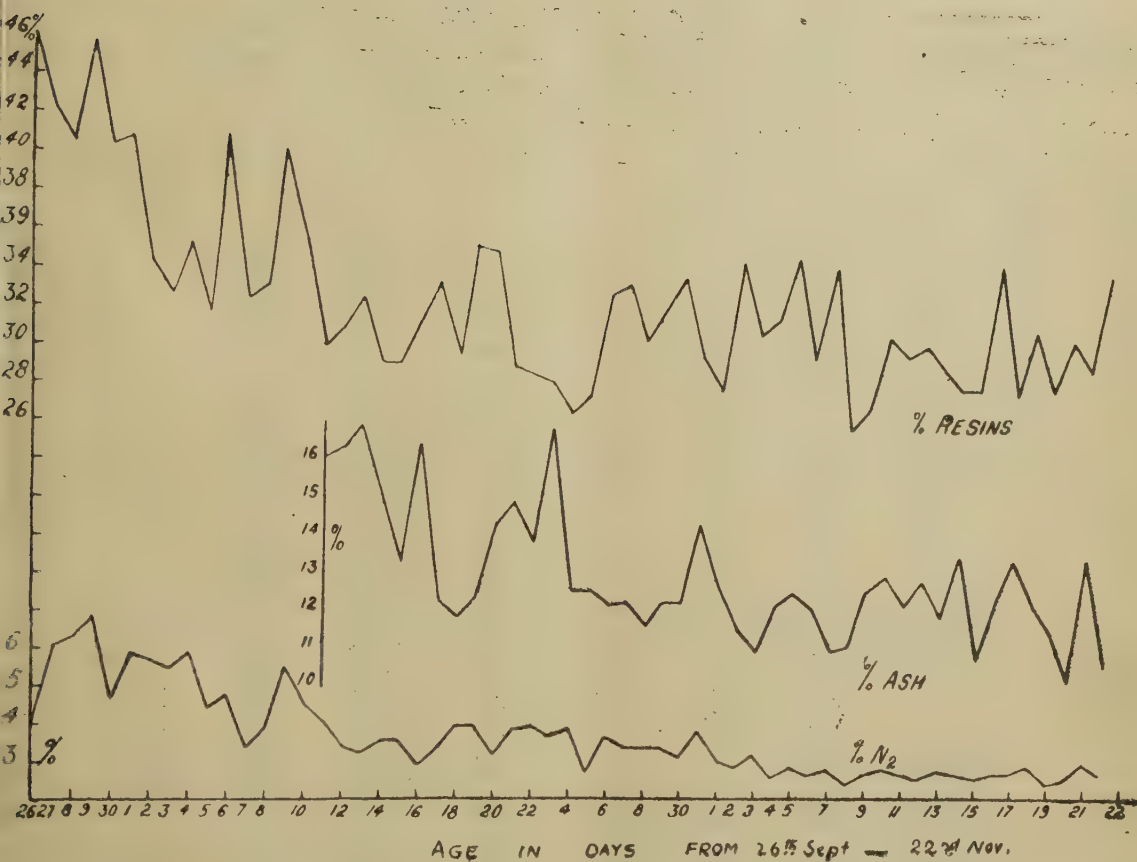


FIG. 1. Percentage of nitrogen, ash and resins of *Cryptostegia grandiflora* seedlings

SUMMARY

A study of the metabolic changes of the inorganic constituents, nitrogen and resins of *Cryptostegia grandiflora* seedlings has been made in relation to air day to day growth till the plants are 62 days

It was found that with polymerisation of the products of photosynthetic activity there was a gradual decrease in the percentages of nitrogen and inorganic constituents while resins fluctuated within

a certain range. An account of an apparatus designed to extract several samples at a time has also been given.

ACKNOWLEDGEMENTS

Our thanks are due to Mr J. P. Anderson, Controller of Rubber, and Major H. R. Walden, Incharge, Wild Rubber Section, Department of Supply, for financial assistance for work on *Cryptostegia grandiflora*.

TABLE I

The growth observations and the results of analyses of Cryptostegia grandiflora seedlings

Rate of growth							Analytical results		
Date when seedlings were pulled out	Age of seedlings in days	Average length of root of 10 seedlings in in.	Average length of stem of 10 seedlings in in.	Average No. of leaves of 10 seedlings	Fresh weight of 10 seedlings in gm.	Dry weight of 10 seedlings in gm.	Percentage of nitrogen	Percentage of ash	Percentage of resins
23-9-43 . . .	1	—	—	—	—	—	No analysis was done		
23-9-43 . . .	2	—	—	—	—	—	Ditto		
24-9-43 . . .	3	—	—	—	—	—	Ditto		
25-9-43 . . .	4	—	—	—	—	—	Ditto		
26-9-43 . . .	5	—	—	—	—	0.0824	4.08	—	46.34
27-9-43 . . .	6	—	—	—	0.38	0.0950	6.14	—	42.24
28-9-43 . . .	7	—	—	—	0.40	0.0690	6.32	—	40.83
29-9-43 . . .	8	2.3	1.3	2	0.39	0.0674	6.872	—	45.48
30-9-43 . . .	9	1.9	1.2	2	0.47	0.0656	4.688	—	40.45
1-10-43 . . .	10	2.5	1.3	2	0.50	0.0726	5.796	—	40.75
2-10-43 . . .	11	2.6	1.3	2	0.54	0.0870	5.686	—	34.67
3-10-43 . . .	12	1.3	1.4	2	0.31	0.0774	5.524	—	32.81
4-10-43 . . .	13	1.5	1.7	2	0.63	0.0780	5.928	—	35.31
5-10-43 . . .	14	2.6	1.6	2	0.95	0.1202	4.436	—	31.84
6-10-43 . . .	15	2.3	1.5	—	0.81	0.1090	4.788	—	41.11
7-10-43 . . .	16	2.6	1.4	2	0.87	0.1010	3.296	—	32.59
8-10-43 . . .	17	2.1	1.5	3	1.15	0.1420	3.758	—	33.23
9-10-43 . . .	18	3.6	1.5	4	0.65	0.1306	5.548	—	40.26
10-10-43 . . .	19	2.3	1.5	4	0.94	0.2006	4.436	—	35.64
11-10-43 . . .	20	4.9	1.3	4	1.26	0.2530	3.972	16.14	30.06
12-10-43 . . .	21	2.9	1.9	4	1.19	0.2376	3.344	16.31	30.90
13-10-43 . . .	22	3.7	1.9	6	1.63	0.3618	3.207	16.88	32.58
14-10-43 . . .	23	3.5	—	4	2.05	0.3394	3.532	—	29.24
15-10-43 . . .	24	3.5	2.2	5	1.16	0.2920	3.452	13.27	29.26
16-10-43 . . .	25	3.1	2.2	6	1.88	0.6268	2.819	16.38	31.37
17-10-43 . . .	26	3.2	2.4	6	1.88	0.3546	3.169	12.97	33.49
18-10-43 . . .	27	3.2	2.5	6	1.92	0.4974	3.882	11.78	29.58
19-10-43 . . .	28	4.1	2.4	6	2.85	0.4538	3.891	12.38	35.48
20-10-43 . . .	29	3.4	2.5	6	2.90	0.4500	3.232	14.34	35.18
21-10-43 . . .	30	3.6	2.7	5	2.97	0.4622	3.760	14.99	29.04
22-10-43 . . .	31	3.5	2.5	6	3.10	0.4942	3.891	13.84	28.54
23-10-43 . . .	32	3.2	2.5	6	2.52	0.3476	3.561	16.90	28.31
24-10-43 . . .	33	3.1	2.9	6	2.88	0.4192	3.768	12.61	26.73
25-10-43 . . .	34	2.9	2.3	6	2.08	0.3104	2.559	12.59	27.66
26-10-43 . . .	35	3.6	2.0	6	2.32	0.4318	3.621	12.19	33.34
27-10-43 . . .	36	4.2	2.6	6	3.1	0.5800	3.282	12.33	33.42
28-10-43 . . .	37	3.7	2.5	6	3.0	0.6200	3.282	11.64	30.62
29-10-43 . . .	38	3.9	2.8	7	3.9	0.7200	3.312	12.33	32.21
30-10-43 . . .	39	3.8	2.5	6	3.55	0.7006	3.141	12.16	33.96
31-10-43 . . .	40	4.8	2.8	8	4.37	0.9520	3.784	14.32	29.80
1-11-43 . . .	41	5.2	2.6	8	4.39	0.8010	2.952	12.66	28.09
2-11-43 . . .	42	5.6	2.9	8	4.23	0.9526	2.845	11.53	34.83
3-11-43 . . .	43	5.2	2.9	7	3.22	0.6674	3.097	10.94	31.04
4-11-43 . . .	44	4.7	3.1	7	4.40	0.7846	2.506	12.07	31.80
5-11-43 . . .	45	4.8	3.0	8	3.40	0.6930	2.793	12.50	34.95
6-11-43 . . .	46	5.8	3.3	9	4.51	0.9458	2.630	12.05	29.82
7-11-43 . . .	47	5.0	3.5	9	4.47	1.2744	2.660	10.88	34.59
8-11-43 . . .	48	6.0	3.5	9	5.17	1.2974	2.399	11.12	26.00
9-11-43 . . .	49	5.6	3.9	10	6.37	1.4318	2.612	12.56	27.35
10-11-43 . . .	50	5.5	3.7	10	5.6	1.2924	2.838	12.85	30.96

TABLE I (concl'd.)

The growth observations and the results of analyses of Cyptostegia grandiflora seedlings

Rate of growth							Analytical results		
Date when seedlings were pulled out	Age of seedlings in days	Average length of root of 10 seedlings in in.	Average length of stem of 10 seedlings in in.	Average No. of leaves of 10 seedling	Fresh weight of 10 seedlings in gm.	Dry weight of 10 seedlings in gm.	Percentage of nitrogen	Percentage of ash	Percentage of resins
-11-43 . . .	51	2.6	3.3	9	5.36	0.9634	2.600	12.22	29.96
-11-43 . . .	52	5.6	3.7	10	7.47	1.4004	2.501	12.76	30.56
-11-43 . . .	53	5.6	3.9	10	8.27	1.5700	2.685	11.83	29.27
-11-43 . . .	54	4.7	3.1	9	4.05	0.8020	2.576	13.37	28.35
-11-43 . . .	55	5.5	2.2	11	10.28	1.9298	2.541	10.76	28.31
-11-43 . . .	56	6.2	3.1	11	6.9	1.2990	2.565	12.27	34.83
-11-43 . . .	57	6.0	4.0	10	11.1	2.3516	2.596	13.37	28.08
-11-43 . . .	58	6.5	3.9	10	6.2	1.8118	2.767	12.24	31.52
-11-43 . . .	59	5.5	3.7	10	6.9	1.5270	2.301	11.35	28.17
-11-43 . . .	60	4.8	3.5	9	7.15	1.4780	2.432	10.09	31.12
-11-43 . . .	61	4.3	3.8	10	5.9	1.5170	2.911	13.36	29.27
-11-43 . . .	62	4.2	4.1	9	6.78	1.5846	2.649	10.62	34.59

N.B. The colour of the root was white, that of the stem and leaves was pink and green respectively after one week germination.

STUDIES ON THE BORON STATUS OF SOME BENGAL SOILS

By M. O. GHANI, M.Sc., Ph.D. (LONDON) and A. K. M. FAZLUL HAQUE, M.Sc.,

Department of Soil Science, Dacca University

(Received for publication on 16 December 1944)

ALTHOUGH the problem of boron nutrition of plants has been receiving increasing amount of attention in its various aspects in the hands of plant pathologists, soil scientists, biological chemists and fertilizer producing concerns in different countries of the world, particularly in America, practically nothing is known about the boron status of Indian soils and the influence of boron on Indian agriculture. In Europe and America, borax has already become a standard ingredient of fertilizers for certain crops and the practice is rapidly spreading with the progress of research in this field.

It is, therefore, a matter of first rate importance from the view point of practical agriculture to ascertain the deficiency or sufficiency of this element in Indian soils and to study the problems arising out of it. Once the deficiency is known to occur, the next steps would be to find out the localities affected, the extent of deficient areas, the various plants affected and the measures necessary to counteract the deficiency. The object of the present work is to throw some light on the boron status of the Bengal soils taken from different parts of the province.

ANALYTICAL METHODS

Determination of boron in soil extracts. In the determination of boron in soil extracts, the quinalizarin colorimetric method of Berger and Truog [1939] was used all through this work. Preparation of colour standards, 98.5 per cent sulphuric acid solution by weight, quinalizarin solution and other reagents required in the determination were made exactly in the same way as was described by the above authors.

Determination of water-soluble boron of soils. After making numerous tests with water at different temperatures and by changing the mode of extraction, it was found by Berger and Truog [1939] that refluxing of the soil-water suspension for five minutes was the best procedure for extracting the water-soluble boron of the soil. The same authors [1940] showed that the results thus obtained gave good correlation with plant response to boron fertilization. De Turk and Olson [1941] showed that water-soluble boron is a fairly reliable measure of the available boron content of soils. This method is now widely used for the determination of available boron of soils and has been adopted in this work as well.

Determination of acid-soluble boron of soils. Richards *et al.* [1942] stated that digestion of a soil with a fairly strong acid would furnish a means for separating the maximum available boron from the soil. According to him, although tourmaline and other boro-silicate minerals will not be affected by strong acids, the boron in organic matter, in precipitated borates or boron in soil colloids should be readily liberated as boric acid by such a digestion and would therefore give a measure of the boron-present in soil which, though not immediately available, would represent the portion available with difficulty. They used 85 per cent orthophosphoric acid as a digesting solution. Dilute acids have also been tried by some workers. Askew and associates [1937, 1938] extracted several New Zealand orchard soils with 0.05 hydrochloric acid and found 0.05 to 0.68 p.p.m. of boron in the extracts. Woodbridge [1940] found that water containing carbon dioxide extracted 0.09 to 0.33 p.p.m. of boron from several soils from British Columbia. Berger and Truog [1940] extracted eight soils with N/10 sulphuric acid both by shaking for 30 minutes and by refluxing for five minutes. From its analogy as an extractant for easily available soil nutrients such as phosphorus and potassium, N/2 acetic acid was used in this work. The extraction was done by refluxing for five minutes and the soil-solvent ratio was the same as in water extraction.

Determination of total boron of soils. In the determination of total boron in soil, the soil must be completely decomposed and fused as it contains highly resistant boro-silicate minerals. Alkaline fluxes, sodium carbonate, potassium carbonate, sodium acid phosphate may be used as a fusion agent. Berger and Truog [1939] found that fusion with anhydrous sodium carbonate followed by addition of sulphuric acid to bring the reaction of the final solution within a pH range of 5.52 to 6.00, hastens

the disintegration of the melt and leaves most of the sesquioxides and silica in insoluble form. The larger amount of sodium sulphate that is formed could be thrown down by the addition of alcohol upto 60 to 70 per cent by volume. The small amounts of non-volatile organic matter usually introduced with the alcohol may be destroyed by igniting the residue obtained after the final evaporation. McHargue and Hodgkiss [1941, 1942] using this procedure obtained satisfactory results for the total boron content of soils. Berger and Truog [1939] method was used in this work for the determination of total boron of soils.

Water soluble boron by shaking method. It was pointed out by Berger and Truog [1939] that refluxing of the soil-water suspension (soil-water ratio 1 : 2) for five minutes was the best procedure for the determination of available boron of soils. Refluxing for periods either shorter or longer than 5 to 10 minutes extracted more boron. They were able to recover completely the soluble boron added to soils by this method. The addition of hot water followed by shaking for 30 minutes did not result in complete recovery. The effect of time of shaking on the available boron extracted was not, however, investigated by them. To see the effect of time on the amount of available boron extracted by the shaking method, the following procedure was adopted.

Twenty gm. samples of soils were weighed into shaking bottles and 100 c.c. of redistilled water added to each. The bottles were then shaken in a mechanical shaker for periods of 15 minutes, 30 minutes, 45 minutes and an hour. After the shaking, the soil was immediately filtered and boron was determined on an aliquot of the filtrate. Six soils representing different soil conditions and containing different amounts of water-soluble boron were selected for this work. The results are shown in Table I.

TABLE I

Effect of time of shaking on the water-soluble boron in p.p.m.

Soil	15 min. shaking	30 min. shaking	45 min. shaking	1 hour shaking	Refluxing for 5 min.
Dacca (No. 1)	0.87	1.00	0.87	0.63	0.63
Barisal (No. 4)	0.63	0.75	0.62	0.40	0.63
Berhampore (No. 10)	0.75	0.87	0.75	0.75	0.45
Badarkhali (No. 13)	1.00	1.12	1.00	0.87	2.00
Hijalbeel (No. 16)	0.75	0.87	0.75	0.62	0.40
Hawar area (No. 18)	0.63	0.75	0.62	0.45	1.12

It will be seen from Table I that the available boron extracted by shaking for 15 minutes is greater than that obtained by refluxing for five minutes in the soils having a low content of available boron, while in the two soils rich in available boron, shaking extracted less boron. It may mean that part of the boron present in the latter is present in a form which is not soluble in water at the ordinary temperature. It will be further seen that as the period of shaking becomes longer, the amount of boron extracted at first increases and then gradually falls off. The maximum amount of boron is extracted at the 30 minutes shaking period. This is true for all the six soils studied. It would appear that along with the depletion of the soil boron, an opposing reaction by which boron is precipitated into insoluble compounds takes place simultaneously and as time progresses the opposing reaction gets the upper hand. This is probably why Berger and Truog [1939] could not recover over the whole of the added boron by shaking for 15 minutes with hot water while refluxing for five minutes effected complete recovery. This precipitation is probably counteracted if the extraction is done by boiling water and hence as the time of refluxing is lengthened, more and more boron (may

be of the more resistant type as well) come into solution. The five minutes refluxing procedure of Berger and Truog was finally adopted in this work for the determination of available boron.

RESULTS OBTAINED

The water-soluble boron, acid-soluble boron and total boron content of 26 samples under study are shown in Table II. pH, organic carbon content and exchangeable calcium content of the soils are also shown in the same table. The soil samples analysed were collected from different parts of Bengal. They were selected for this investigation as representative of various types of soils found in different regions of the province. They covered both the acid and alkaline ranges of pH. In the case of soils obtained from the district farms and other experimental farms, whenever possible, samples were taken from both unmanured plots and from plots fertilized with different kinds of artificial fertilizers and organic manures. Some samples were taken from typical lowland areas where the soil remains submerged under water for four to five months and are specially adapted to the cultivation of 'boro' paddy.

TABLE II

Results obtained (boron expressed as p.p.m.)

Soil No.	Description	pH	Water-soluble boron	Acid-soluble boron	Total boron	Percentage of organic carbon	Percentage of exchangeable calcium
1	Dacca Farm	5.1	0.63	0.45	33	0.907	0.130
2	Bankura Farm, unmanured	5.2	1.37	1.12	55	0.887	0.080
3	Bankura Farm, manured	5.2	0.87	0.75	100	0.731	0.132
4	Barisal Farm, manured	8.4	0.63	0.45	43	0.731	1.877
5	Barisal Farm, unmanured	7.6	1.62	1.37	100	0.868	2.065
6	Midnapore Farm, manured	8.2	1.12	0.63	100	0.936	0.064
7	Midnapore Farm, unmanured	8.0	1.12	0.87	100	0.868	0.040
8	Rajshahi Farm, manured	8.2	1.12	0.87	90	0.960	1.527
9	Rajshahi Farm, unmanured	8.2	0.87	0.62	100	0.956	0.882
10	Berhampur Farm, unmanured	7.0	0.45	0.62	100	0.936	0.066
11	Red Soil I, Vowal area	8.0	0.45	0.63	100	0.780	0.065
12	Red Soil II, Vowal area	8.0	0.75	0.63	100	0.907	0.098
13	Badarkhali (Chittagong) 'Kosch soil'	5.2	2.00	1.75	..	0.751	0.064
14	Sutahata (Midnapore) Flood area	8.0	1.12	1.37	..	0.887	0.140
15	Hathhazaria I (Chittagong)	8.0	0.45	0.75	..	1.180	0.088
16	Hijalbeel (Murshidabad) Lowland	8.0	0.40	0.87	..	1.073	0.177
17	Tista Silt (Rangpur)	7.6	0.45	0.75	..	0.468	0.370
18	Hawar area I (Mymensing)	5.4	1.12	0.87	..	1.580	0.168
19	Hawar area II (Mymensing)	6.4	0.50	0.45	..	0.919	0.140
20	Daulatpur Farm (Khulna)	8.0	1.12	0.87	..	1.082	0.263
21	Jessore Farm	6.4	1.12	0.87	..	0.539	0.177
22	Bankura, Highland	5.2	0.75	0.50	..	0.406	0.066
23	Suri Farm	8.0	0.87	0.75	..	0.410	0.564
24	Rangamati (Chittagong)	4.4	0.50	0.45	..	0.662	0.045
25	Hathhazaria II (Chittagong)	4.4	0.75	0.63	..	0.254	0.077
26	Paralbari (Midnapore) Flood area	5.0	1.37	1.12	..	1.034	0.241

DISCUSSION OF RESULTS

Effect of pH on availability of boron. It will be seen from Table II that the available boron content of the 26 soils under study ranges from 0.4 p.p.m. to 2.0 p.p.m. The average comes to 0.9 p.p.m. The pH of the soils ranges from 4.4 to 8.4. In the 12 acid soils the available boron comes, on an average, to 0.95 p.p.m., whereas, in the 14 soils having their pH above 7.0 and extending up to 8.2, the average of available boron comes to 0.86 p.p.m. There is also considerable variation in the available boron of soils having the same or nearly the same pH. For example, soil Nos. 6, 7, 8, 9, 11, 12, 14, 15, 16, 20 and 23 having their pH at 8.0 or in the neighbourhood, contain all amounts ranging from 0.40 to 1.12. These results show that the amount of available boron present in a soil is determined by factors other than its pH. The nature of the parent material, the extent of weathering and leaching, the system of cropping and the chemical composition of the soils may be assumed to play a large part in determining the available boron content of a particular soil. The results obtained by previous workers are of somewhat conflicting nature. Haas [1937] stated that a deficiency of boron is most likely to occur on alkaline or overlimed soils while Brandenburg [1932] and Cook [1940] reported that the heart-rot of sugar beets was common on acid as well as on alkaline soils. Cook and Millar [1939] observed that there were so many cases of deficiency on acid soils and non-deficiency on alkaline soils that other factors than pH value were of greater importance. According to them supply of calcium and organic matter, and soil texture were most important in affecting boron availability. Purvis and Hanna [1939] reported that majority of boron deficient areas in America occurred on acid soils.

Effect of organic matter and exchangeable calcium on boron availability. Cook and Millar [1939] believed that permeability of the sub-soil horizon, supply of calcium and organic matter and soil moisture were the most important soil factors affecting boron availability. They showed that there was a correlation between the appearance of heart-rot in sugar beets and active calcium content of the soils. Even when the effect of pH was minimized by placing the soils into acid and alkaline groups, there was evidence to show that the soils on which heart-rot of sugar-beets occurred were higher in active calcium content than were the soils on which heart-rot did not occur. Purvis [1939] found that tolerance of soils to application of borax was correlated fairly well with the organic matter content and exchange capacity. Less injury was produced by borax in soils having a high organic carbon content and high exchange capacity than in a soil having a lower carbon content and exchange capacity. The results obtained in this work, how-

ever, show that there is no apparent relation between the availability of boron in the soils and their organic carbon or exchangeable calcium content.

Acid-soluble boron. The results show that in the majority of soils, the amount of boron dissolved by N/2 acetic acid is less than water-soluble boron. Out of the 26 soils, only in six soils the acid-soluble boron is higher than the water-soluble boron. All these soils are alkaline in reaction, having a pH of 8.0 in four cases. But as there are other alkaline soils in this series which have shown a decrease, no generalization is possible on the basis of pH. The ratio of water-soluble boron to acid-soluble boron falls within a very narrow limit; in most of the soils the ratio varies from 1.1 to 1.4, which shows that in the majority of soils acetic acid extraction method will give a relative measure of the available boron content of soils.

Total boron. Total boron was determined accurately only in a few samples; in most of the remaining soils the quantity was much above 100 p.p.m. and could not be determined accurately as the colour developed was too blue to be compared with the standards. Much of this total boron is present as particles of resistant rocks and minerals residual from the decomposition of the parent material. The most commonly identifiable boron mineral in soils is tourmaline which is a complex aluminium boro-silicate of iron, magnesium or other bases and which contain 3.1 per cent of boron. The boron present in this form is highly unavailable to plants. The results obtained by previous workers show that many soils contain high quantities of total boron though the available portion is usually very small. Bertrand and Silverstein [1939] analysed 24 soils from Europe and Africa for their total boron content and found it to vary from 7 to 50 p.p.m., 75 per cent of them between 10 to 30 p.p.m. In 10 soil samples, Luchetti [1938] found the total boron to range from 20 to 100 p.p.m. Rogers and associates by spectroscopic analysis, estimated as much as 100 to 500 p.p.m. of boron in certain soils from Florida.

Distribution of available boron by areas. In the absence of any well defined classification or grouping of the Bengal soils, it is difficult to say with any amount of precision whether the deficiency or non-deficiency of boron is connected with any particular soil type of the province. It may, however, be observed that the soils taken from the Chittagong district are low in their available boron content. The soil Nos. 15, 24 and 25 taken from that area contain 0.45, 0.50 and 0.75 p.p.m. of available boron respectively. Soil No. 13 (Badarkhali) taken from the coastal tract of Chittagong is the only exception. This soil contains the highest amount of available boron, namely, 2 p.p.m. This soil which goes by the name of 'Kosch' soil, was

cluded in this series because of its high infertility which cannot be ascribed to any of the known causes. The result obtained here is interesting because the soil is found to contain a very high amount of available boron which may fall within the border line of toxicity and thus be partly responsible for the unproductivity of the soil. The soils from the district of Midnapore which are subjected to occasional flooding by sea water reveal great similarity in their available boron content, the quantity being much higher than those of the Hattagong area. Thus soil Nos. 6, 7, 14 and 26 from this area contain 1.12, 1.12, 1.12 and 1.37 p.p.m. of available boron respectively. This high figure must be due to inundation of the area by sea water. It has been shown by Moberg and Harding [1933] that the concentration of boron in sea water is about 4.5 p.p.m. Richards *et al.* [1942] have stated that irrigation water may add boron to the soil of the cultivated areas. From a comparison of water soluble boron of a number of irrigated and non-irrigated soils, they found that the irrigated soils contained 2.84 p.p.m. of water soluble boron on an average as compared with an average of 0.87 p.p.m. in the non-irrigated soils. According to them much of the boron added by irrigation water is retained by the soil in a readily soluble form, thereby considerably increasing the water-soluble boron content. The soils from Barisal, Khulna and Jessore (Nos. 4, 5, 20 and 21) forming part of the saline tracts of the Sundarbans also show a similarly high figure for available boron (average 1.12 p.p.m.). The three uncultivated soils from Dacca, Hijalbeel and Tista (Nos. 1, 16 and 17) are fairly low in available boron. Similar is the case with the red soils from the Vowal area. The average boron content of the soils taken from the interior, namely, Jessahi, Bankura, Berhampore and Suri shows an average which is nearly equal to the general average of the available boron in the 26 soils studied. There does not seem to be any consistent difference between boron availability of manured and unmanured soils of the same locality.

The results obtained here do not, however, allow any inference to be drawn as to whether the concentration of boron usually present in Bengal soils falls within the deficiency or toxic concentration of the element. Such an inference is only possible when extensive field trials are made on different types of soils and with different kinds of crops and the laboratory results are calibrated. From the results obtained by various workers in other countries, it appears that the concentration of boron below which deficiency symptoms will appear, lie within a range of 0.25 to 0.4 p.p.m. and that boron will begin to show its toxic effect as it approaches a concentration of 2 p.p.m. From this point of view, the Bengal soils studied do not seem to indicate either

excessive or deficient concentration of available boron.

Boron present in organic combination. Boron is added to the soil with every addition of vegetable and animal matter and with decay and decomposition of the organic residues, it will be liberated in inorganic forms. As the decomposition of the organic matter is constantly going on in the soil through the agency of micro-organisms, the soil organic matter may be looked upon as a reserve for the available boron. It was thought that determination of the organic boron content of soils might have useful information to a study of the boron status of soils. No method has yet been devised for estimating the boron that is present in soils in organic combination.

It is known that when soil material is ignited, organic matter is destroyed and therefore the mineral nutrients are converted into inorganic forms. The increase in the soluble constituents of a soil caused by ignition may therefore be assumed to be derived from the mineralization of the soil organic matter. If the extraction is done under identical conditions, the increase would represent the portion which has come from the organic matter by its destruction or in other words the increase will represent the organic form of the element. This principle has been utilised in determining organic boron of the soils.

Twenty gm. of soils were weighed in a porcelain basin and the basin was placed inside an electric muffle. The muffle was adjusted at about 600°C. and the ignition was carried out for an hour. The sample was then cooled, transferred to the distilling flask and boiled under a reflux condenser with 40 c.c. of distilled water for about five minutes. Boron was then determined on an aliquot of the extract. From this was subtracted the amount of boron extracted from the unignited soil under identical conditions of extractions. The difference was taken to represent the organic boron of the soil. The results are shown in Table III.

TABLE III
Organic boron in p.p.m.

Soil No.	Water-soluble boron of fresh soil	Water-soluble boron of ignited soil	Increase due to ignition
G1	0.63	1.87	1.24
G2	1.37	1.62	0.25
G3	0.87	0.87	0.00
G4	0.63	0.63	0.00
G5	1.62	2.37	0.75
G6	1.12	1.87	0.75
G7	1.12	1.62	0.50
G8	1.12	1.75	0.63
G9	0.87	1.37	0.50

TABLE III (concl'd.)
Organic boron in p.p.m.

Soil No.	Water-soluble boron of fresh soil	Water-soluble boron of ignited soil	Increase due to ignition
G10	0.45	0.75	0.30
G11	0.45	0.75	0.30
G12	0.75	0.87	0.12
G13	2.00	2.75	0.75
G14	1.12	2.25	1.13
G15	0.45	2.12	1.67
G16	0.40	2.12	1.72
G17	0.45	2.12	1.67
G18	1.12	2.00	0.88
G19	0.50	2.12	1.62
G20	1.12	2.12	1.00
G21	1.12	1.12	0.00
G22	0.75	2.12	1.37
G23	0.87	2.12	1.25
G24	0.50	1.87	1.37
G25	0.75	2.63	1.87
G26	1.37	1.37	0.00

It will be seen from Table III that in most of the soils ignition has increased the amount of water-soluble boron. In some cases the increase is as high as 1.87 p.p.m. In four soils there is no increase, showing that there is little boron present in organic combination whereas in half a dozen soils the increase is small as compared with others.

SUMMARY

Twentysix soil samples taken from different parts of Bengal were analysed for their available boron content by the quinalizarin colorimetric method of Berger and Truog. The available boron content of these soils ranges from 0.4 p.p.m. to 2.00 p.p.m., the average being 0.9 p.p.m. The pH of the soils has little effect on the availability of soil boron. In the 12 acid soils the average comes to 0.95 p.p.m. whereas in the 14 soils having their pH above 7 the average is 0.86 p.p.m. of boron.

In most of the soils boron soluble in N/2 acetic acid is less than the amount in water-soluble form. The ratio of water-soluble boron to acid-soluble boron falls within the range of 1.1 to 1.4 in most cases.

There is no apparent relation between the availability of boron and the organic matter or exchangeable calcium content of the soils studied.

The soils taken from the district of Chittagong are generally low in available boron whereas the soil taken from the district of Midnapore which are subjected to occasional flooding by sea water contain a much higher percentage of boron. The soils from the saline tracts of Sundarbans also show a similarly high figure.

The amount of boron extracted by shaking with water is less than that obtained by refluxing for five

minutes in the soils having smaller amounts of available boron while shaking extracted less boron from the soils which are rich in available boron. The amount of boron extracted by water at first increases with the increase in the period of shaking reaching a maximum at the 30 minutes shaking period after which it falls off slowly.

Ignition of the soil in most cases increased the amount of water-soluble boron, sometimes by as much as 1.87 p.p.m. The difference between the water-soluble boron of the ignited and the fresh soil has been assumed to represent the boron present in the soil in organic combination.

REFERENCES

- Askew, H. O. and associates (1937). The boron status of New Zealand fruit soil. *N. Z. J. Sci. Tech.* **18**, 789-96
- (1938). Effect of borax top dressing on boron status of soil and fruit. *N. Z. J. Sci. Tech.* **20**, 74A-78B
- Berger, K. C. and Truog, E. (1939). Boron determination in soils and plants using the quinalizarin reaction. *Industr. Engng. Chem. Analyt. Ed.*, **11**, 540-45
- (1940). Boron deficiencies as revealed by plant and soil tests. *J. Amer. Soc. Agron.* **32**, 297-30
- Bertrand, G. and Silverstein, L. (1939). Sur la Teneur Du Sol En Bore. *C. R. Acad. Sci. Paris.* **208**, 1453-6
- Brandenburg, E. (1932). The heart and dry rot of beets due to boron deficiency. *Angew. Bot.* **14**, 194
- Cook, R. L. (1940). Factors influencing availability of boron in soil and its distribution in plants. *Soil Sci.* **50**, 209-17
- De Turk, E. E. and Olson, L. C. (1941). Determination of boron in some soils of Illinois and Georgia. *Soil Sci.* **52**, 351-7
- Haas, A. R. C. (1937). Boron deficiency effects in general appearance to bark symptoms of psorosis in citrus. *Soil Sci.* **43**, 317-25
- Luchetti, G. (1938). Ricerche Su Terreni Della Zona Boreale Di Larderello, 1-11 Boro Nel Terreno E Nella Plante Pisa Unis. *Ann. Fac. Agrar. (n. s.)* **14**, (177) 195
- McHargue, J. S. and Hodgkiss, W. S. (1941). Report on Iodine and Boron. Association of Agricultural Chemists **24**, 518-20
- (1942). Report on Boron and Fluorine in Soils. Association of Agricultural Chemists **25**, 311-13
- Purvis, E. R. and Hanna, W. J. (1939). The influence of overliming upon boron deficiency. *Amer. Fertil.* **9**, (8), 5-7, 24
- Richards, R. et al. (1942). Boron distribution in soils and related data. *Tech. Bull. U. S. Dep. Agric.* No. 797
- Woodbridge, C. G. (1940). The boron content of some Okangan soils. *Sci. Agric.* **20**, 257-65

SOME OBSERVATIONS ON THE GENETICS OF LINSEED

By T. S. SARNIS, B.A. (HONS.), D. SC., F. A. SC., I. A. S., Economic Botanist (Oilseeds) and T. R. MEHTA, B. Sc., Assoc. I. A. R. I., Research Assistant to the Economic Botanist (Oilseeds) to Government, U. P., Cawnpore

(Received for publication on 13 February 1945)

LINSEED ranks second in importance among the oilseeds of the United Provinces, the first place being held by *Brassicæ*. The area under linseed in the United Provinces is about 900,000 acres, which is roughly $2\frac{1}{2}$ per cent of the total cropped area of the province. Relative to the linseed acreage in India, the crop occupies about 22 per cent of the area in the United Provinces which comes next only to the Central Provinces and Berar which claim 28 per cent of the area. But in the matter of production, the United Provinces ranks first, contributing 30.9 per cent of the total produce, followed by Central Provinces and Berar which contribute 17.9 per cent.*

Breeding work on linseed was started at the Botanical Research Farm, Cawnpore, as early as 1924-25 when a collection of varieties of Indian linseeds and foreign flax varieties was made. In subsequent years crosses were made between the indigenous and exotic varieties, with a view (1) to combine rust

resistance of the flax varieties with the higher yield and oil content, earliness and bigger seed size of the indigenous types and also (2) to isolate flax like hybrids suitable for conditions obtaining in the United Provinces. One of the crosses made with the latter object has given a number of hybrid forms which while retaining, and some of them exceeding, straw length of the flax parent possess a suitable duration and adaptation to local conditions. The exotic flax types were never a success due to the poor germination, long duration and defective seed setting. The extracted hybrids are free from these shortcomings. Two of these, viz. Fx3 and Fx6, are now available for multiplication.

The above cross also provided an opportunity for the study of inheritance of colour character of the flower. The parents were English White and C. P. White: their characters are recorded in Table I.

TABLE I

The characters of C. P. White and English White

Parents	Flowers	Petal	Anther	Seed
C. P. White . .	Large, open . .	White with pink tinge and violet streaks	Light blue . .	Dull white
English White . .	Medium sized, not fully open	White with very faint bluish tinge	Salmon . .	Brown

The types were crossed in 1928-29, and later again crossed in 1938-39 to confirm the observations made earlier.

F₁ Generation. The F₁ plants had, unlike the parents, blue corolla, and the anthers were light blue and the seeds brown in colour.

F₂ Generation. The F₂ generation was studied for the segregation of corolla colour. Four main phenotypic classes were observed, viz. two parental classes, one class like the F₁ and a new white-petalled class. They have been termed *C P W* (to represent the corolla colour of C. P. White), *E W* (to represent the corolla colour of English White), *Blue* (the F₁ class) and *W* (the new white-petalled class). The frequencies were as follows (Table II).

The values of X^2 in the two cases are, respectively, 1.91 and 1.87. The corresponding values of *P* are 0.603 and 0.604 respectively, which shows that the fit is good in both the cases.

F₃ Generation. The data obtained in F₃ were in close agreement with the expected segregations of the hybrids and are not presented here. The frequencies given by the segregating F₃ families were recorded and the fit in every case was very close.

The behaviour of corolla colour in inheritance is similar to that recorded by Shaw, Khan and Alam [1931]. In a cross between two linseed types, 1 and 12, possessing corolla colour similar to that of C. P. White, and English White respectively, they secured in F₂ a dihybrid segregation. However, they observed a deficiency in the double recessive phenotype which they ascribed to some lethal combination of two factors. It is quite possible that the size

* See *Report on Marketing of Linseed in India* Published by the Manager of Publications, Delhi

TABLE II

The frequencies of phenotypic classes in F₂ generation

	F2 Classes				Total
	Blue	CPW	EW	W	
<i>English White</i> x <i>C.P. White</i>					
Observed	1504	471	493	174	2642
Expected on 9 : 3 : 3 : 1 basis	1486.1	495.4	495.4	165.1	2642
<i>C. P. White</i> x <i>English White</i>					
Observed	1443	489	497	178	2607
Expected on 9 : 3 : 3 : 1 basis	1466	489	489	163	2607

of F₂ population studied by them, viz. 342 plants, was not large enough to give the expected strength of the double recessive phenotype, and that with a larger population no such deficiency might have been observed. In the above cross between C. P. White and English White, no such deficiency was observed. However, the inheritance can be explained according to the theory applied by Shaw, Alam and Khan. Employing the genic symbols used by them we can represent the constitution of the corolla of the two parents as follows:—

C. P. White—BB CC dd EE FF.

Eng. White—bb CC DD EE FF.

B is a factor which acts with C to produce pink colour in the petal.

C is a factor for colour in the petals and acts with B to produce pink.

D is a factor which modifies pink to lilac. In the absence of B and the presence of E, it causes a faint tinge of blue.

E is a factor which intensifies colour in the petal.

F is a factor which dilutes pink colour in the petal.

The F₁ of the above cross had blue petals and had the constitution BbCCDdEEFF. The F₂ had the four phenotypes in the proportion 9:3:3:1 as follows:

9 BCDEF	3 BCdEF	3 bCDEF	1 bCdEF
Blue like F ₁	White like C. P. White	White like Eng. White	White with no blue tinge

ANTHER COLOUR

Among the numerous hybrid families raised in different years segregations for anther colour, seed colour, height, duration and habit were observed and were recorded in some cases. Thus in 1940-41 counts were made in 49 segregating families for an-

ther colour for the phenotypes 'light blue' and 'salmon' colours. The segregation was mono-genic, giving in every case the ratio 3:1 with the usual variation. The totals for the 49 families are:

	Light blue	Salmon
Observed	2291	771
Expected	2296.5	765.5
Ratio	3	1

The fit is good.

SEED COLOUR

Counts of brown and fawn seeded segregates in 19 families gave the following frequencies:

	Brown	Fawn
Observed	771	249
Expected	765	255
Ratio	3	1

The difference in seed colour thus appears to be due to a single gene, a fact which agrees with the findings of Ali Mohammad and Khan [1941] who ascribe the brown seed colour to the interaction of two factors G and M and fawn colour to the operation of M alone and absence of G.

DISCUSSION

The similarity between the results presented above and those described by Shaw, Khan and Alam [1931] and by Ali Mohammad and Khan [1941] is significant. This similarity would only mean that although the material which the different workers used represented widely different agricultural and commercial varieties of this crop, it possessed similar, if not

identical, genes for colour of petals, anthers and seeds. It would appear, therefore, that the genic constitution of linseed is comparatively simple in spite of the great variability shown by this crop. Unfortunately the records on the genetics of linseed are meagre and whether the genic constitution is more complex than what appears from recorded work, is a question which must be left to the future.

SUMMARY

The inheritance of petal colour in a cross between two pure lines of linseed was studied and was found to be governed by five genes, the parents differing from each other in two genes only. The results agree

with those obtained by Shaw, Khan and Alam, viz. a dihybrid segregation into four phenotypes in the ratio 9 : 3 : 3 : 1. The double recessive phenotype, however, did not show any deficiency in number as that observed by the other workers.

The inheritance of anther and seed colour was also studied and was found to be monogenic.

REFERENCES

- Ali Mohammad and Khan, A. R. (1941). Some breeding investigations of linseed in the Punjab. *Indian J. agric. Sci.* **11**, 432-45.
 Shaw, F. J. F., Khan, A. R. and Alam, M. (1931). Studies in Indian Oilseeds. V. The inheritance of characters in Indian linseed. *Indian J. agric. Sci.* **1**, 1-57.

METHODS OF COMPUTING PEST INCIDENCE

By HEM SINGH PRUTHI, M. SC. (PUNJAB), PH. D. and SC. D. (CANTAB.), F.R.A.S.B., F.N.I. and S. PRADHAN, D. SC. (LUCK.), Assistant Entomologist (Population Studies). Imperial Agricultural Research Institute, New Delhi

(Received for publication on 2 January 1945)

E. M. N.

(With one text-figure)

As expected an insect pest generally attracts the serious attention of an economic entomologist only when at least once its attack assumes such devastating severity that the problem of its immediate control overshadows all other considerations. Naturally therefore, under such conditions enough thought cannot be devoted to such matters of scientific accuracy as those of methods employed for estimating intensity of attack or measuring pest incidence, etc. But when these so to say emergency methods are recorded in print they naturally are followed up by subsequent workers who overlook the emergency character of those methods. Due to this circumstantial sequence for which nobody can be said to be really responsible, we find today in the literature on economic entomology that a large number of defective methods of computing and comparing pest incidence are being continued by worker after worker. The aim of the present article is to invite the attention of entomologists to some typical examples showing the nature of flaws existing in the present day common methods. An effort has been also made to offer an easy correction for the main flaw. Of course, it is by no means intended to belittle the works of previous investigators; the apology for the critical analysis presented herein lies in the fact that as result of the labours of the same previous workers now economic entomology has reached that proud stage when it requires all the accuracy of exact sciences and when at least glaring flaws in its technique must be examined and corrected.

COMMON METHODS OF COMPUTING INCIDENCE

As will be clear from the actual references some of which are cited later on, the incidence of both pests and parasites is generally recorded in the form of 'Percentage Values'. The pest is recorded as percentage of the material liable to be damaged; for example in the case of cotton bollworms it is a common record that bollworm incidence was say 50 per cent, meaning thereby that 50 bollworms were found per 100 buds and bolls examined or 50 per cent of the bolls and buds examined had bollworms. Again in the case of sugarcane borers we often come across statements like 'top-borer incidence was 10 per cent', 'stem-borer incidence 15 per cent' and so on. Similarly parasite incidence is recorded as percentage of the host insect examined.

Some authors have made slight deviations from the above general practice; some of these deviations afford comparatively a little better method, some mean essentially the same expressed in different terms and there are some others which at first thought appear to constitute improvements but are actually much worse. For example, some workers on cotton bollworms have expressed incidence as 'number of bollworms per 25 plants' which is the same thing as percentage incidence with respect to the number of plants instead of percentage incidence with respect to the number of buds and bolls. As will be shown later this deviation is a little for the better. Workers on leaf-miners and leaf-suckers like aphids and whiteflies express incidence as 'number of insects per

leaf' which is the same things as percentage incidence with respect to the number of leaves. Other workers on the same pests express the incidence as 'number of insects per unit area of leaf surface'. As will be shown later this is an example of deviation which is apparently more scientific than incidence expressed as number per leaf but is actually much worse.

THE CHIEF FLAW AND ITS CORRECTION

The chief flaw in all the above methods is that they ignore the fundamental axiom that the scale used for measurements must be constant if the measurement is to be reliable. When the incidence of a pest is recorded as percentage of the material liable to be damaged, the quantity of the damageable material becomes the scale against which the pest population is measured. Thus the number of buds and bolls becomes the scale in the case of bollworms, the number of canes in the case of cane-borers, the number of leaves or area of leaf-surface in the case of leaf-miners, aphids, whiteflies, etc. Similarly when we express the incidence of a parasite as percentage of the host parasitized, the number of the host insect becomes the scale against which the parasite population is measured. Now these scales are obviously not constant. In nature both the quantity of damageable plant material in the case of pests as well as the quantity of parasitizable insect host material, in the case of parasites practically vary from zero to infinity in the same season. The plant material, i.e. crop is absent, then it is sown, and it germinates, then it gradually grows in size and quantity and is finally harvested away; similarly the insect host material, i.e. pest is at first practically absent, then it makes its appearance, and its number gradually increases and then decreases again. This is an illustration of only seasonal variation in these scales; there are variations due to several other factors as will be clear shortly. Thus the scales with which we measure the incidence of pests and parasites are subject to such wide fluctuations that the measurements, i.e. 'percentage incidence' values, cannot be expected to be reliable or comparable at all. Let us take a concrete example of bollworm percentage incidence. Suppose at the time of first observation the field contains 100,000 buds and bolls and there are 10,000 bollworms distributed in the field, then if we conduct sampling in the usual way we will get 10 bollworms per 100 buds and bolls examined, i.e. we will record 10 per cent incidence. Further suppose that when the same field is examined after some time for second observation the number of buds and bolls in the field has increased to 200,000, but the number of bollworms has remained unchanged at 10,000, then in the second observation the sampling will give only 5 bollworms per 100 buds and bolls examined, i.e. we will record only 5

per cent incidence. The comparison of these two observations, i.e. 10 per cent and 5 per cent incidence will ordinarily lead us to conclude that bollworm activity has decreased in the field due to some reason although obviously it has not been actually the case. These observations will lead us to an erroneous conclusion simply because the scale used in measuring the incidence has not been constant, i.e. the number of buds and bolls has changed. That such erroneous conclusions are actually arrived at in practice due to misleading nature of 'percentage incidence' is amply proved by the following actual observations in the field: While studying the population fluctuation of spotted bollworm of cotton, we have been recording the total number of bollworms per acre, the total number of buds and bolls per acre, and also the percentage incidence of bollworms with respect to the number of buds and bolls. All the three sets of data for one complete season are graphed in Fig. 1. From this figure it will be clear how the percentage incidence often gives absolutely wrong idea. For example, starting from *e* in Fig. 1, it will be seen that the number of bollworms rises or at least remains constant up to the point *f*, but since the number of buds and bolls has considerably risen from *e* to *f* the percentage incidence shows a steep fall. Again after the point *n*, as the number of buds and bolls is rapidly decreasing the percentage incidence is showing rapid rise though actually there is little rise in the actual number of bollworms. In fact it is needless to point out the other discrepancies which exist: where the trend of change is indicated rightly the rate of change is hardly correct. These are the difficulties met with even when we compare the data from week to week only; the flaw becomes all the more glaring when we compare the data from year to year. Thus when we compare our own data on the basis of 'percentage incidence' of spotted bollworm with respect to buds and bolls for the three seasons (i. e. 1941-42, 1942-43, 1943-44) it appears beyond doubt that infestation was entirely different from season to season, the highest percentage incidence in the three successive seasons being 10 per cent, 5 per cent and 21 per cent respectively; but when we compare the population per acre for the same three seasons we find that the population was almost the same, the highest level reached in the different seasons being 23,000, 20,000, and 22,000 respectively. Thus the percentage incidence data alone would have erroneously indicated undue apprehension in 1943-44 and an unduly false sense of security in 1942-43, and would have set the entomologists thinking about the cause of these differences.

Probably recording of 'percentage incidence' has come in vogue under the impression that the quantity of the host material remains so huge as compared with that of the parasite that the former can be

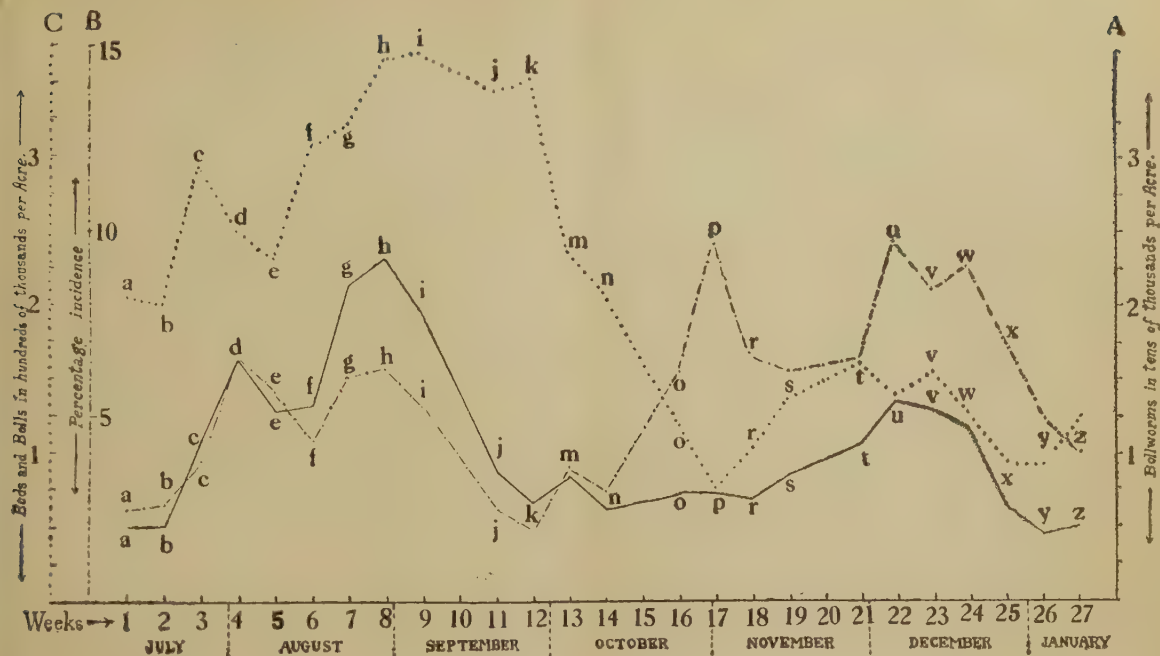


FIG. 1—The weekly fluctuation in the population of the spotted bollworm of cotton at Delhi during 1941-42.

- A. The weekly fluctuation in the actual population per acre, the numbers 1, 2, 3 on its scale representing tens of thousands;
- B. The weekly percentage incidence of bollworms with respect to buds and bolls, the numbers on its scale representing percentage figures;
- C. The weekly population of buds and bolls per acre, the numbers on its scale representing hundreds of thousands.

The letters *a* to *z* represent the points on the three graphs corresponding to the numbers along the abscissa which represent serial numbers of the weeks, i.e. 1. week ending on 12th, 2. on 19th, 3. on 26th July, 4. on 2nd, 5. on 9th, 6. on 16th, 7. on 23rd, 8. on 30th August, 9. on 6th, 10. on 13th, 11. on 20th, 12. on 27th September, 13. on 4th, 14. on 11th, 15. on 18th, 16. on 25th October and so on.

taken as constant like the level of the sea. The fallacy of this impression will be clear from the theory of biological control in which effort is made to reduce the population of the host with the help of parasite. But surprisingly even in work on biological control 'percentage incidence' method is being used by many authors. Obviously the quantity of the host cannot be taken to be constant otherwise the experiment will be against the presumption. If the host material is constant it cannot be controlled and if it can be controlled it cannot be constant.

DEFECTIVE METHODS ILLUSTRATED

We are giving below a few typical illustrations from entomological literature showing the magnitude of error likely to creep in the results in case the fundamental axiom pointed out above is ignored. These illustrations will also show that the arguments advanced against the common method of computing percentage incidence are also applicable with greater or lesser force to the various modifications of that common method mentioned before.

1. Taking examples from Indian literature only the extensive work on pink bollworm right from the early papers of Fletcher and Misra [1921], Willcocks [1921], Ballard [1922], up to most recent papers and reports, is based on 'percentage incidence' only. For instance in a paper entitled 'Incidence of *Platyedra gossypiella* in relation to climate (1926-31)' by Haroon Khan [1938] both the regional variation as well as the seasonal variation have been studied with the help of 'percentage incidence' only and on the basis of this study Punjab has been divided into four zones wherein 'percentage incidence' has been shown to vary between different limits. Now if the same zones are compared as regards number of bolls per acre there is obviously some difference between these four zones and this difference also must have resulted into some difference in the values of 'percentage incidence' even if the population of pink bollworms per acre were to be the same in all the zones. If the difference in these four zones as regards number of bolls per acre is in the same direction as the difference in population of bollworms per acre, then the

calculation of 'percentage incidence' as reported by the author must have masked the actual difference in bollworm activity in these zones. Therefore the differences shown by the author's data on percentage incidence must be regarded as underestimates. On the other hand, if the differences in bolls per acre and bollworms per acre are in opposite direction then the differences shown by this author's data must be regarded as overestimates. It will be instructive to consider in this connection that the yield of cotton per acre probably decreases as we travel from western part of the Punjab to eastern Punjab and if this decrease in yield is due to decreasing number of bolls per acre from west to east then, even if the bollworm population be uniformly distributed in the province, we should still find mathematically a progressive increase in percentage incidence from west to east as reported by the author, but this progressive increase will be due to mathematical principle and not due to any ecological law so far as bollworm distribution is concerned. Of course we do not mean to contend that the whole of the difference in percentage infestation between different zones is due to difference in boll production but the data given in the paper under reference do not show as to how much the difference in boll production is responsible for difference in 'percentage incidence'. This factor of boll production would not have interfered with the estimate if the data would have been given in terms of population per acre.

2. Again in a more recent paper by Afzal Husain and Haroon Khan [1940], the Indian and American varieties of cotton have been compared with respect to pink bollworm infestation and here also the same flaw exists since only 'percentage incidence' has been taken as the criterion. Now if the *desi* and American varieties are compared as regards the number of bolls per acre there is bound to be some difference and this difference will itself create some difference in the values of 'percentage incidence' even if the bollworm number per acre be the same in *desi* and American varieties. Probably the number of bolls per acre may be less in the case of American than in the case of *desi* varieties (although the yield per acre may be more in American due to larger size of individual bolls), and the higher 'percentage incidence' in American cotton may be partly due to this fact. Our contention holds good even if the case is just the reverse, i.e. even if the number of bolls per acre may be more in American than in *desi* varieties in which case the difference shown by the authors between these varieties should be regarded as underestimates.

3. The monumental work on spotted bollworms carried out from 1923-31 by Deshpande and Nadkarny [1936] is based on 'number of bollworms per 25 plants' which as explained before, is the same thing as 'percentage incidence' with respect to plants.

This criterion contains the flaw already discussed by us although to a lesser degree. Such data are comparable from week to week if they are taken from the same field because the number of plants does not change much from week to week in the same field but such data are not comparable from year to year when crop changes and the number of plants also changes. Even if population per acre remains the same during different seasons, the season in which the germination remains comparatively poorer gives larger number of bollworms per plant or per 25 plants because the same population distributes itself on a lesser number of plants; similarly better germination produces the reverse effect. Our own data referred to above show that although the highest level of population per acre reached during the three seasons (1941-42, 1942-43, 1943-44) varied only as 23,000, 20,000 and 22,000, yet due to differences in population density of plants per acre during different seasons, the maximum value of number of bollworms per 100 plants fluctuated during the same period as 280, 110 and 384 respectively. Thus the work of Deshpande and Nadkarny contains detailed weekly data on number of bollworms per 25 plants for six consecutive seasons (1925-31) and the maximum number of bollworms per 25 plants recorded for these different seasons are 87, 144, 78, 130, 204 and 220. These data without being converted into population per acre cannot give any comparative idea as to the extent to which the different seasons were favourable or unfavourable for bollworm multiplication because the number of plants was not uniform during all the six seasons.

4. Very recently Trehan [1944] has studied incidence of whitefly of cotton by calculating number of immature stages of the whitefly per square inch of leaf area. This criterion is even worse than 'percentage incidence' based on the 'number of leaves' as leaf area is more variable than leaf number. Thus, for example, suppose in a field there are 1,000 leaves and suppose the average area per leaf is 4 sq. in. in the young crop of cotton, the total leaf-area of the field would be 4,000 sq. in. Now the whole population (say 20,000) of immature stages of whitefly will be distributed over an area of 4,000 sq. in. and a sampling will give five insects per sq. in. or twenty insects per leaf. Now suppose at the time of the next observation the insect population has remained practically unchanged at 20,000 but the number of leaves has increased from 1,000 to 2,000 and also the size of leaf has increased from 4 sq. in. to 8 sq. in. Now the total leaf-area of the field will be 16,000 sq. in. over which the population of 20,000 insects will be distributed. Therefore the sampling will show 20,000/16,000, i.e. 1.25 insects per sq. in. of leaf-area or 20,000/2,000, i.e. ten insects per leaf. Thus although the population

of the insect in the field has remained unchanged the number per leaf will show a decrease to one-half, i.e. from 20 to 10 whereas the number per sq. in. will show a decrease to one-fourth, i.e. from 5 to 1.25. Thus neither the number per leaf nor number per unit area of leaf surface can give the correct picture but the latter is more incorrect than even the former although at first thought the latter appears to be more scientific. On the basis of this defective criterion, i.e. 'number per sq. in.' Trehan has compared the different varieties of cotton with respect to whitefly infestation but the flaw will be evident if one just considers how much the different varieties of cotton differ in their leaf area. Thus on page 57 (*op cit.*) the following conclusion is reported: 'Taking the entire season the whitefly attack on *desi* cotton was significantly higher than on the American cottons during both the years.' Now probably the total leaf area of *desi* cotton per acre may be less than that of American cotton and therefore even if the population of whitefly per acre be the same in the two varieties the number per sq. in. may come out more in *desi* cotton than in American cottons, but this difference in number per sq. in. will not show any varietal preference shown by whitefly or varietal susceptibility of cotton. No such difficulty would have arisen if the different varieties of cotton were compared by comparing the population of whitefly per acre in the different varieties. This author has also reported in the same paper his conclusions regarding the effects on whitefly infestation of various other factors, e.g. irrigation, pH value, cultivation conditions, etc. and all these conclusions are based on 'number per sq. in.' It is needless to explain further that the effects of these factors on the leaf area of cotton are bound to have either exaggerated or masked the effect on whitefly infestation.

5. The list of papers containing the flaw can be increased to any length. We have very briefly analysed above just a few cases merely as illustrations. In the reports from various provinces the cane borer attack is given as 'percentage incidence' with respect to number of canes and tillers which vary from season to season and variety to variety. We may add that similar mistakes are not uncommon in papers published in other countries. Wolcott and Martorell [1943] have expressed the incidence of *Trichogramma* throughout as percentage of its host number. Smyth [1938] has expressed the incidence of sugarcane borer as well as that of its parasite in 'percentage values'. Box [1938] has compared plant crop and ratoon crop of sugarcane for borer infestation and this comparison is also based on 'percentage incidence' only. The incidence has been reported to be lower in ratoon than in plant crop. Now it is not clear whether the number of borers was actually less in ratoon or the 'percentage

value' has come out to be less in ratoon due to there being larger number of canes in ratoon crop than in plant crop.

CONCLUSIONS

From the foregoing it should be clear that if we want reliable data on incidence of pest or parasite it should always be calculated with reference to some fixed scale. So far as the agricultural crop pests and the parasites of those crop pests are concerned, the unit area of the land on which that crop is grown provides a fairly good and constant scale. Thus taking the case of bollworm incidence, if instead of estimating 'percentage incidence' we estimate population per acre and suppose we get 10,000 bollworms per acre in the first observation, then this number cannot change in subsequent observations by any alteration in the condition of the crop unless the number of bollworms actually increases or decreases. It is hardly necessary to point out that population per acre can be easily computed by simultaneously estimating by ordinary sampling procedure the number of insects per plant as well as number of plants per acre and then applying simple rule of three. If the nature of the insect under study allows, population per acre can also be calculated by directly counting insects on small units of land area. In cases wherein this change advocated above is found impracticable on other considerations and the incidence has to be recorded as percentage of the material liable to be damaged then care should be taken also to record along with it the average population per acre of the host material so that the incidence of the pest or parasite may be ultimately referred back to the fixed scale of unit land area whenever comparison with future record is desired.

REFERENCES

- Afzal Husain, M. and Haroon Khan, M. (1940). *Indian J. Ent.* **2**, 45-57
 Ballard, E. (1922). *Agri. Res. Inst. Pusa Bull.* No. 133
 Box, H. E. (1938). *Proc. 6th Cong. Int. Soc. Sug. Tech.*, 239
 Deshpande, B. P. and Nadkarny, N. T. (1936). *Imp. Coun. Agric. Res. Sci. Monogr.* 10
 Fletcher, T. B. and Misra, C. S. (1921). *Agri. Res. Inst. Pusa Bull.* No. 105
 Haroon Khan, M. (1938). *Indian J. agric. Sci.* **8**, 191-214
 Smyth, E. G. (1938). *Proc. 6th Cong. Int. Soc. Sug. Tech.*, 367-77
 Trehan, K. N. (1944). *Indian J. agric. Sci.* **14**, 53-63
 Willcocks, F. C. (1921). *Agri. Res. Inst. Pusa Bull.* No. 107
 Wolcott, G. N. and Martorell, L. F. (1943). *J. econ. Ent.* **36**, 460-64

SOME FIELD OBSERVATIONS ON THE PRESENT CYCLE OF DESERT LOCUST (*SCHISTOCER CA GREGARIA* FORSK.) IN SIND

By M. HAROON KHAN, B.Sc.(LOND.), A.R.C.S., Assistant Entomologist, Imperial Agricultural Research Institute, New Delhi (Formerly Assistant Entomologist, Sakrand, Sind) E.M.N.

(Received for publication on 11 April 1945)

THE present cycle of the desert locust, which started in 1940, is remarkable in more than one respect. For the first time this country was forewarned of the locust invasion [Pruthi, 1940], and it was also definitely established that the heavy infestation of the summer, 1941, originated mainly from the swarms of foreign origin [Pruthi, 1941]. A significant feature of this cycle was that it afforded the entomologists of the country an opportunity of directing and supervising the offensive against this pest in its home—the permanent breeding grounds; during previous cycles the battle against the pest had to be waged after it had invaded other areas, because breeding grounds had not been located in this country. From August-October, 1941, the Thar Desert, which constitutes the south-eastern portion of Sind, was the centre of one of the severest infestations ever recorded, and one great and incessant battle took place in this tract over a wide front of several hundred miles.

The battle against the pest seemed already a lost one in some parts of this desert, towards the middle of August, 1941, when the writer was sent by the Government of Sind to give technical assistance. Two years later, in 1943, he was again sent to the same area with a similar purpose. During the course of very extensive touring of several weeks in 1941 and again in 1943, it was possible to study under natural conditions the behaviour of the pest when it was at its worst.

THAR DESERT

The Thar Desert, a part of the so-called Great Desert of India, occupies an area of about ten thousand square miles. It comprises the entire Chachro, Diplo and Mithi talukas, great part of Nagar Parkar and Umerkot talukas, and part of Khipro and Sanghar talukas. It is a tract of rolling sand hills which rise up to 150 ft. and run mainly from north-east to south-west.

The annual rainfall varies from 7 to 14 in., and almost all of it is received between June to October. Cultivation depends entirely on rain. *Bajri* (*Pennisetum typhoideum*) is very extensively grown, sometimes mixed with *til* (*Sesamum indicum*) and *moth* (*Phaseolus acontifolius*). Besides these, *guar* (*Cyamopsis psoraloides*) is almost the only other crop. The people, however, depend more on the produce of their herds of cattle and flocks of sheep and goats than they do on their cultivated fields. The locust swarms arrive in this tract either just before or after the first few showers of rain.

IMMIGRATION OF SWARMS

During July and August, 1941, many swarms of yellow locust passed over parts of Sind; they generally came from north-west and went towards south-east. On two occasions swarms were observed flying at right angles to the direction of the wind while its velocity exceeded seven miles an hour. The first observation was made at Sakrand, on 28 July 1941. The swarm was flying very low and had segregated into thousands of units. Each unit consisted of 20-100 locusts, and all of them were following the same direction a short distance from one another. The locusts flew only short distances, may be 30-150 yd. and were then forced down to the ground by strong wind. After a few minutes they again took to wings and were forced down again a short distance ahead. This went on for over half an hour till the entire swarm passed away. A similar case was observed on 2 August 1941, at a village, Kak, 20 miles away from Khipro in Thar Desert. This type of flight was also observed by my colleagues twice.

The regular migrations of this locust in the solitary phase, eastwards from Baluchistan to Rajputana in May and June, and back from Rajputana to Baluchistan in October and November, has been fully established [Rao, 1940]. During May and June the general direction of wind, which is usually over 5-6 miles per hour, is from south and south-west and the pest flies partly against the wind because the direction of flight is from north-west, i.e. from Baluchistan to Rajputana side. Probably the pest mainly flies while the velocity of wind is low.

OVIPOSITION

In Sind, extensive egg-laying took place in the Thar Desert after the commencement of monsoon in 1941 and 1943, and to a lesser extent in other parts of Sind during spring, 1943. The Thar Desert received over 7 in. of rain during July and August, 1941 and the entire desert tract was very suitable for egg-laying. Swarms of locust invaded this desert from about the middle of July to the end of August and were observed pairing and egg-laying under diverse conditions—in the fields with standing crop, in fallow land with or without vegetation, near the base of perennial bushes and rarely in thick annual vegetation. The mounds of sand near the base of perennial bushes were preferred.

During February and March, 1943, egg-laying was reported from some non-irrigated tracts of Larkana

Dadu districts. This area is ordinarily most suitable for egg-laying because the soil is very hard, and there is little or no rainfall during winter and spring. The heavy floods during July, 1942, in the North Sind had, however, flooded this barren tract and several swarms laid eggs in an area stretching over 60 miles.

During July, 1943, there were good showers of rain in parts of the Thar Desert, and egg-laying took place over a wide area. It was interesting to observe that a swarm had moved from north to south in Chachro and Umerkot talukas, over a distance of about 80 miles, and laid eggs all over the area. The egg-laying was heavy in the north but it steadily decreased southward and, in areas further south it, did not take place. The hatching of eggs, as was expected, took place first, on 10 August, in the north and on or about 20 August in the extreme south.

EMERGENCE OF VERMIFORM LARVAE

Vermiform larvae were frequently observed in the process of casting their overalls. They were found crawling around the emergence holes at a distance of about three inches. After casting the overalls the hoppers moved singly in all directions, and took shelter on the nearest bushes or annual plants.

FORMATION OF BANDS

The primary factor in the formation of bands appears to be, as pointed out by Uvarov [1928], the disposition of egg pods in more or less dense groups and congregation of hoppers from the beginning of their life. Besides this factor, Uvarov is of the opinion that the response to light and warmth also play an important part. Over a large stretch of thousands of square miles in the Thar Desert, conditions of light and warmth are very similar and it is doubtful if these factors have any direct influence on the formation of bands.

One other factor which assists in the fusion of bands is the physical feature of the breeding areas. When two or more bands near each other, it was noticed, that they accidentally fused while climbing a sand dune. Before climbing a dune the bands locate gradual slopes, and either in the process of locating or in following the same path, the bands fused. Bands coming down from two or more sand dunes to a common valley fused likewise. Special mention may be made of the formation of bands and their fusion noticed in a village in Chachro taluka between 14 and 16 August, 1943. On 14 August several long streaks of 1st stage hoppers, only 2-3 days old, were noticed on 4-5 sand dunes. The following morning streaks of hoppers from these and some other dunes were observed coming down to a wide valley continuously for about seven hours. These streaks fused, as the day advanced, and assumed the form of a regular band. Again, on 16th several loose bands were observed coming from the same

direction and they congregated more and more in the valley.

COMPOSITION OF BAND AND ITS SIZE

Bands composed of hoppers of one or two instars were generally observed, but on several occasions bands of all the five instars, mostly of 3rd-5th instars, were also noticed. In almost all the big bands, some hoppers, hardly one per cent, of the solitary phase were found; and in bands of 5th stage a number of adults of gregarious or solitary phase were always present, marching with them and behaving like hoppers.

The size of bands varied considerably; while some bands occupied an area of a few hundred square feet, others extended over several square miles. Generally bands of 1st and 2nd stage hoppers were small and those of 3rd to 5th stage hoppers were large. Special mention may be made of two exceptionally big bands, one of 1st stage hoppers and the other mainly of 3rd to 5th stage hoppers. The former was noticed on 15 August 1943, and was about half a mile long and a quarter of a mile in width. Ballard, *et al.* [1932] have stated that bands of 1st stage hoppers make local migrations within hatching grounds, but this band and several others were observed migrating in body from their breeding areas. The other band was seen near Islamkot between 10 and 14 September, 1941. The width of the band varied from two to four miles and it continuously passed near and partly through the town for five days, in a chain broken only at the night time. Its length could not have been less than 10 miles.

Associated with the size of a band is the question of its density. In the morning and evening bands were generally very dense, most of the hoppers being hardly half an inch apart, but during the day the hoppers spread out and became an inch or more apart. At times it was not possible to say as to where one band ended and another started, because either hoppers of the fusing bands had not come quite close to each other or bands in heavily infested areas had segregated, after exhausting the vegetation, into many units that moved in all directions in the face of starvation. Some bands were also secondarily split up into several bands owing to the passing of cattle or a large number of hoppers being left behind for moulting. It is thus evident that a band is never composed of the same hoppers but is picking up and leaving behind a large number of hoppers during course of its journey.

Further, it is suggested tentatively that the term band may be used for those hoppers that follow the same direction, occupy an area of 200-400 sq. ft. (depending upon the stage) while the distance between most of the hoppers be not more than two inches.

MOVEMENT OF BANDS

Bands of hoppers were observed moving almost throughout the day, from about an hour after sun-rise to an hour or so before sun-set and on a few occasions, even before sun-rise or after sun-set. Once only, on 5 September, 1941, three bands of 5th stage hoppers were noticed moving quite fast, 2-4 hours after sun-set, on a full moonlit night.

Bands were generally observed to follow the direction of wind; two exceptionally big bands referred to above also followed the direction of winds. Ballard *et al.* [1932] found similar correlation between the direction of wind and the movement of bands. Once on their march, the bands moved up and down the sand dunes that fell on the way, following always the path of least resistance.

The speed of hoppers varied greatly during the day. In the morning and evening, their movement amounted to slow crawling, while during the greater part of the day it varied between crawling and jumping. Ballard *et al.* observed that some hopper bands covered a distance of about five miles in a day. It was possible to make careful observations once when it was estimated that a band of 1st stage hoppers covered a distance of about three-fourth of a mile in a day.

BREAKING OF BANDS AND FORMATION OF SWARMS

The breaking up of 5th stage hopper bands takes place gradually as those about to moult leave the band and prepare for ecdysis. The bands thus thin down. As this process goes on the proportion of adults increases. In big bands, the proportion of adults was observed to be only 1-5 per cent, in medium size bands 5-20 per cent and in small bands 20-50 per cent. Two very small bands seen on 5 September in a highly infested area consisted of almost cent per cent adults. La Baume [1918] also observed the latter type of bands in Moroccan locust and called them 'adult bands'. It is thus evident that the occurrence of adults bands is brought about by the gradual segregation and not as a result of large number of adults emerging simultaneously. Freshly emerged adults show gregarious instinct and join any passing band.

It could not be definitely ascertained as to how long the adults remained associated with the hoppers, probably not more than 2 or 3 days. Thereafter they segregated, as a rule individually. Their segregation from 'adult bands' was not observed. Adults, which were able to fly, left the band in various ways; either by remaining on bushes and plants in the morning, when hoppers resumed their march, or by climbing on bushes and plants that fell on their way, or by joining any swarm that passed over-head.

The segregation of adults on bushes appears to be the first step in the formation of a primary swarm, a

condition resembling somewhat the emergence of hoppers after 'intermediate moult' and their going on to the bushes. The number of adults found on bushes varied with the population of full grown hoppers. The formation and hovering of small primary swarms near the place of their origin was witnessed 4-7 days after their emergence in September, 1941. As these swarms flew others joined them. After about a week of their emergence, loose swarm were observed, and their flight appeared to be a sustained one—the distance covered at a time being a mile or more.

THE BEHAVIOUR OF SWARMS

The swarms commenced migrating from the Thar Desert a little earlier than usual in 1941 because of fairly warm weather in September and October. An approximate idea of the number of days which these swarms took to reach to other parts of the country was formed by following the reports of swarm movement. The summer brood adults originated on a very large scale between the first week of September and the middle of October, 1941, and some of these passed over parts of Nawabshah district between 17 and 19 September. Thus they took about a fortnight, after their emergence to cover a distance of 150-200 miles and reach adjoining tracts of Sind. In the last week of September and throughout October, large number of swarms were reported from various parts of Sind. These probably originated during September and first fortnight of October and also took about a fortnight to leave their place of origin.

The influence of temperature on the flight of swarms has not been studied. Swarms of desert locust were, however, observed to fly at different temperatures. The actual temperature of the air at the level at which they were flying or the temperature of the body has not been determined, but the two extremes of temperatures in shade when swarms were noticed at Sakrand were 110°F. on 28 July, 1941 and 55°F. on 11 January, 1942.

In the evening, just before sun-set, swarms settle down on trees or bushes and resume their flight 2-3 hours after sun-rise the next morning. A swarm which settled at about sun-set in a forest near Sakrand on 17 October, 1941, was watched on the following morning from about 7 A.M., the time of sun-rise. The day was a bright one, but up to 8-30 hours the locusts remained on the top branches of *kandi* (*Prosopis spicijera*) trees and showed no sign of activity. Just after 8.30 hours, they became active and started flying downwards, either to lower branches of the trees or to other low growing trees, and then flew down to the ground. There was a continuous stream of locust to the ground up to 10 hours. Those that came to the ground first started flying soon after 9 hours, in the form of a thin swarm,

and an almost continuous swarm kept departing till 9.30 hours.

The temperature recorded in a thermograph, hardly a mile away, varied from 72°F. at 7 hours to 80°F. at 10 hours, while the relative humidity fell down from 76 per cent to 42 per cent during the same period.

FOOD OF HOPPERS AND ADULTS

Bhatia [1940] studied the food preference of the literary phase of this pest in Thar-Rajputana tract and found that all the common plants were eaten; most of these plants were also eaten by hoppers and sometimes by adults of the gregarious phase. Even leaves of *ak* (*Calotropis gigantea*), which Bhatia found were not accepted after 24 hours of starvation, were eaten by hoppers on 24 September 1941 when the day was very hot and the infestation very heavy. There seemed no difference between the food taken by hoppers and adults, but the latter showed special preference for ripe or nearly ripe grains of *bajri*.

Further, some very juicy fruits, such as watermelon (*Citrullus vulgaris*) and musk melon (*Cucurbita moschata*), were found completely consumed; while on another occasion very dry products, such as bark and some outer portion of plants of rape (*Rassica napus*), its seed pods and grains, which contain very little moisture, were seen eaten. This is not very surprising because even wool, as shown by Afzal Husain and Mathur [1936], is eaten to obtain moisture.

Cannibalism was observed to be very common during the third week of April, 1943, in Dadu district. Several hundred hoppers, mostly in the act of molting, and freshly emerged adults were found mercilessly eaten, usually from posterior end, by other hoppers. This phenomenon was neither observed nor was ever reported from the Thar Desert. The primary cause for cannibalism, as shown by Uvarov [1928], appears to be thirst. In Dadu district the vegetation was dry and the days very hot when this phenomenon was noticed, while in the Thar the vegetation was green and the days mostly cloudy during the period of infestation.

NATURE AND EXTENT OF DAMAGE

The damage caused by the locust in the Thar Desert was two-fold. They damaged the crop and the pasture lands. In 1941 the infestation was extremely heavy and crops and pastures suffered very much. In 1943, on the other hand, infestation was comparatively mild, and the damage was mainly done by hoppers.

During 1941 at least 50 per cent young crops were destroyed by hoppers in Diplo and Mithi Talukas. The damage done in other parts of Thar, though not so marked at first, became very severe when swarms of pink locusts ate away almost all

mature grains. Taking this desert as a whole, the damage to crops in 1941 can safely be put down to three-fourth of the produce. Besides, all the herds of cattle which came for grazing from neighbouring areas had to be taken either to less affected tracts of the desert or some other area; this caused great hardship. No rough estimate for 1943 is possible because parts of the desert could not be visited.

ROLE OF BIRDS IN LOCUST CONTROL

Afzal Husain and Bhalla [1931] studied the bird enemies of this pest in the Punjab and emphasized the invaluable service they rendered by exterminating thin swarms and destroying hoppers that escaped onslaught by man. During the infestation of 1943, the role of some birds, especially Rosy Pastor, in these two directions was most clearly visible.

The following birds, recorded as enemies of locust by Afzal Husain and Bhalla, were observed in the Thar Desert during the period of locust infestation, August-September, 1943:

- (1) Indian Jungle Crow (*Corvus macrorhynchos*);
- (2) Common India House-Crow (*Corvus splendens*);
- (3) Indian Tree-pie (*Dendrocitta vagabunda*); (4) Red-Vented Bulbul (*Malpastes cafer*); (5) Indian Robin (*Saxicoloides fucata cambaiensis*); (6) Fantail Fly Catcher, probably White-Browed (*Leucocirca aureola*); (7) Great (Indian) Grey Shrike (*Lanius excubitor lahtora*); (8) Rufous-backed Shrike (*Lanius schach erythronotus*); (9) King Crow (*Dicrurus macrocerus*); (10) Rosy Pastor or Rose-coloured Starling (*Pastor Roseus*); (11) Common Myna (*Aceridotheres tristis*); (12) Indian House-Sparrow (*Passer domesticus Indicus*); (13) Franklin's Crested Lark (*Galerida cristata*); (14) The Blue-Jay or Indian Roller (*Coracias benghalensis*); (15) Indian Hoopoe (*Upapa epop orientalis*); (16) Laggar-Falcon (*Falco jugger*); (17) Common Pariah Kite (*Milvus migrans govinda*); (18) Common Pea Fowl (*Pavo cristatus*); (19) Northern Grey Partridge (*Francolinus pondicerianus*).

Most of these birds were seen eating hoppers and some of them also adults. Of the above mentioned birds only six, viz. House Crow, Indian Grey Shrike, Rosy Pastor, Common Myna, Indian House-Sparrow and Common Pariah Kite were very common. None of the birds except Rosy Pastor attacked the pest in flocks. This bird was the commonest and probably destroyed more locusts than all other natural enemies put together.

Rosy Pastor is a regular visitor of Thar. It generally comes during September-October when *bajri* crop matures and feeds on grains. During 1941 and 1943 flocks of this bird came at least one month earlier, soon after swarms of yellow locust started coming, and probably came in much larger numbers than in previous years. These flocks were noticed feeding exclusively on hoppers of all the

stages and adults, in almost all the infested areas. The flocks generally consisted of 20-30 birds, but on a few occasions as many as 100 birds were observed. While attacking bands, usually large number of flocks perched together on a few bushes or trees near them.

The role of this bird in reducing the very severe infestation of 1941 could not be fully evaluated, but in 1943 it was quite appreciable. From about the middle of August, 1943, flocks were observed feeding on hoppers, almost from the time of their emergence, in places wide apart; and the flocks increased in number and size as the time advanced. In spite of the good organization set up well in time, during 1943, adults started emerging, in some places up to 10 per cent of the hoppers population, from about the first week of September in Chachro taluka. Almost all of these and in addition a large number of 5th stage hopper bands were completely destroyed by this bird, and no swarm emerged from this desert tract. A number of supervisors of heavily infested areas frankly admitted that they could not, but for the invaluable help of this bird, effectively destroy, with the labour at their disposal, the pest in time. While in mildly infested areas control parties were disbanded a week or more earlier than expected because of the havoc wrought by this bird.

It was difficult to form any definite idea about the population of Rosy Pastor, but on one occasion, while passing through a heavily infested area of about 3 miles $\times$ $\frac{1}{4}$ th of a mile, on 12 September, 1943, the writer saw not less than fifty thousand birds.

The Common Pariah Kite was seen only once in very large numbers—not less than five thousands in one square mile. They were eating hoppers and freshly emerged locusts on the ground and bushes. They, however, attacked the bands individually and not in the form of a flock.

Some other birds, (1) White Cheeked Bulbul (*Malpastes leucogenys*), (2) Ashy Crowned Finch-Lark (*Eremopteryx grisea*), (3) White-backed Vulture (*Pseudogyps begalensis*), (4) Tawny Eagle (*Aquila rapax*) and several types of Doves, were also found, but they were not observed feeding on locusts and are neither recorded as their enemies.

SUMMARY

The observations made on the behaviour of the desert locust during the present cycle, mainly in the Thar Desert, are described.

In their east-ward migration in summer months, swarms may fly at right angles to wind when the wind velocity exceeds even seven miles per hour.

The physical features of the Thar Desert, besides other factors, play an important part in the initial formation of a band or in the fusion of two or more

bands. Bands composed of hoppers of one or two stages are generally found but at times they are composed of three or even all the five stages.

The size of bands varies considerably; bands composed of young hoppers are smaller than those of older hoppers. Small clusters of hoppers, occupying less than 200-400 sq. ft. should not be called bands. Freshly emerged adults are found in bands of 5th stage hoppers and move with them for some time. Bands follow the main direction of wind; in going up or coming down a sand dune, they follow the path of least resistance. Bands composed of hoppers of 5th stage break gradually. The proportion of freshly emerged adults increases with the decrease in size of these bands, and often 'adult bands' are formed.

Adults segregate from bands individually and rest on bushes. About a fortnight after their emergence, they may leave their place of birth and cover a distance of two hundred miles or more.

Pink locusts and hoppers eat almost all desert plants, the former show special preference for grains of *bajri*. Cannibalism was observed only under hot and dry conditions.

A large number of birds eat hoppers and adults. Of these, Rosy Pastor alone attacks the pest in flocks, and is a very important agent in reducing the severity of infestation.

ACKNOWLEDGEMENTS

This paper has been mainly written during the tenure of my present appointment and I am deeply indebted to the Imperial Entomologist for giving me all facilities in its compilation. I am also very grateful to Mr M. Afzal Husain and Dr Taskhir Ahmed for kindly going over the manuscript and giving me valuable suggestions and to Mr B. W. Budd, Collector, Thar Parkar, for giving me the names of the birds he observed in Thar Desert during August and September, 1943.

REFERENCES

- Afzal Husain, M. and Bhalla, H. R. (1931). Some birds enemies of the desert locust (Punjab). *Indian J. agric. Sci.* **1**, 609-19.
- and Mathur, B. C. (1936). Why the locusts eat wool. *Indian J. agric. Sci.* **6**, 263-7.
- Ballard, E., Mistikawi, A. M. and Zoheiry, M. S. El. (1932). The desert locust in Egypt.—*Ministry of Agric. Egypt Tech. & Sci. Serv. Bull.* No. 110.
- Bhatia, D. R. (1940). Observations on the biology of desert locust in Sind-Rajputana desert area. *Indian J. Ent.* **2**, 187-92.
- La Baume, W. (1918). (Quoted from Uvarov, B. P. Locusts and Grasshoppers, 1928)
- Rao, Y. R. (1940). Some observations on the periodicity of locust invasion in India. *Indian J. Ent.* **2**, 193-9.
- Pruthi, H. S. (1940). A fresh cycle of the desert locust in India. *Indian J. Ent.* **2**, 241.
- (1941). Progress of the locust cycle of 1940. *Indian J. Ent.* **3**, 335.
- Uvarov, B. P. (1928). *Locusts and Grasshoppers*—Imp. Inst. London, 1928.

STUDIES ON *EARIAS* SPECIES (THE SPOTTED BOLLWORMS OF COTTON) IN THE PUNJAB

V. THE HOSTS AND HOST-PREFERENCE OF *EARIAS CUPREOVIRIDIS* WLK., *E. FABIA* STOLL. AND *E. INSULANA* BOISD.

By M. HAROON KHAN, B.Sc. (LOND.), A.R.C.S., Assistant Entomologist, Imperial Agricultural Research Institute, New Delhi, LADHA RAM MOHINDRA, B.Sc., GANDA RAM SHARMA, B.Sc., and M. ABDUL GHANI, B.Sc. (AGRI.)

(Received for publication on 15 May 1945)

EARIAS FABIA and *E. insulana* have been known as serious pests of cotton in India for forty years [Lefroy, 1906, 1909]. *E. cupreoviridis*, is a pest of cotton in China [Li, 1937], but is not known to attack cotton in this country, although it is found on other Malveous plants. Fletcher [1917, 1919], Fletcher and Misra [1919], Afzal Husain [1925, 1929], Chopra [1928], and Deshpande and Nadkarny [1936] have recorded the host plants of these species in various parts of India, but there is no precise information with regard to the plants on which the different species subsist. Sometimes vague statements, based on insufficient data, have been made. Richards [1924], for instance, states that all the three species are found on *Abutilon indicum* and cotton. No other worker has so far recorded *E. cupreoviridis* from either of these plants in India. Precise information concerning the food plants of these insects is of very great importance, because the destruction of alternative hosts during the close season, has been recommended as the only effective method of the control of *Earias* in Egypt [Dudgeon, 1916; Gough, 1919], South Gujrat [Deshpande and Nadkarny, 1936] and, by the senior author, for the Punjab. To ascertain fully the part played by the different host plants in the carry-over of these insects, and the plants preferred by each species, detailed observations were made for three years, 1934-36, at Rohtak, Ludhiana, Sialkot, Lyallpur and Multan, representing the various cotton growing tracts of the Punjab. Caterpillars were obtained from flower-buds and pods of various host plants at 7-14 days interval throughout the season and bred in the laboratory.

DISCUSSION OF DATA

Of the 18 or so host plants recorded from various parts of India, the following 11 plants were observed to harbour *Earias* spp. in the Punjab :

1. *Abutilon indicum*.
2. *Althea rosea*.
3. *Hibiscus cannabinus*.
4. *Hibiscus esculentus*.
5. *Malva parviflora*.
6. *Malva sylvestris*.
7. *Malvestrum tricuspidatum*.

8. *Sida cordifolia*.
9. *Sida humilis*.
10. *Sida rhombifolia*.
11. *Urena lobata*.

The distribution of the species was studied on the first eight of these plants. The material obtained from the last three plants was not sufficient for drawing any conclusion.

Abutilon indicum. It is a perennial weed, usually found in waste land and is very common in south-eastern, central and sub-mountainous regions of the Punjab. Except during the severe spell of summer, May-June, it remains green practically throughout the year, but bears flower-buds and pods mainly during February-April and September-November. In moist or shady places it flowers and fruits throughout the greater part of the year, except from about the middle of May to the end of August. Table I gives the number of moths bred from this plant during different months. The moths bred were invariably *E. insulana*. Even during the months of September or October when *E. cupreoviridis* and *E. fabia* were very common on other host plants, not a single moth of these two species emerged from the large collections made from this plant.

Abutilon spp. have been observed as the most favourite hosts of *E. insulana* by King [1918], and Bedford [1931] in Sudan, and their use as trap-crop has been recommended. It may be that *Abutilon* spp. are the original food plants of *E. insulana*, and this view is supported by the fact that one of us collected from a species of this plant a large number of caterpillars of *E. insulana*, during summer 1942, in the Thar Desert, some 70-100 miles from the nearest cotton fields. Further, in countries, such as Iraq and Sudan, where cotton was introduced *E. insulana* was one of the pests observed soon after its introduction, which shows that this insect was present on some wild plants, probably *Abutilon*.

Malva parviflora. It is a common annual weed found in very moist and shady places, such as gardens, banks of canals and grassy plots. It bears flower-buds and pods from about the end of January to about the middle of May.

TABLE I

Number of Earias insulana moths bred from Abutilon indicum

	Rohtak			Ludhiana			Sialkot			Lyallpur	Multan	
Year	1934	1935	1936	1934	1935	1936	1934	1935	1936	1934	1935	1936
January . . .	..	..	179	..	..	..	..	..	7	..	..	..
February . . .	..	..		..	14	..	..	7		..	..	..
March . . .	..	108	260	..	122	38	..	8	6	71	..	..
April . . .	92	238	355	4	197	95	220	24	53	117	9	..
May . . .	42	10	265	87	38	..	134	79	72	..	46	45
September . . .	..	..	74	372	38	56	158	16	84	..	13	3
October . . .	..	..	77	53	80	28	216	134	88	..	3	..
November . . .	}	3	152	116	28	12	168	161	66	..	17	..
December . . .												

TABLE II

Number of Earias insulana moths bred from Malva parviflora

	Ludhiana			Sialkot		Lyallpur
Year	1934	1935	1936	1934	1936	1935
March . . .	..	39	23	18	7	..
April . . .	..	25	12	..	136	11
May . . .	34	15	..	..	11	..

This plant is rarely attacked during the months of January or February, the part of the season when it is flowering. Of the 325 moths bred from *M. parviflora*, all were *E. insulana*. It is possible that this plant escapes the attack of the other two species of *Earias* because they are rarely found during the period when it flowers.

Malva sylvestris. It is a rare plant which was found growing wild in some moist places at Ludhiana. It bears flower-buds and pods mainly from April to July. Only *E. insulana* moths were bred from the collections made (Table III).

TABLE III

Number of Earias insulana moths bred from Malva sylvestris

Period	No. of moths
June 1934 . . .	15
May 1935 . . .	13
June 1935 . . .	16

Hibiscus cannabinus. It is grown for fibre, often around sugarcane or cotton fields in some parts of the Punjab. It is usually sown in May or June, and bears flower-buds and pods from August to November.

Of the 166 moths bred, all were *E. insulana*.

TABLE IV

Number of Earias insulana moths bred from Hibiscus cannabinus

Station	Period	1934	1935	1936
Sialkot	October . . .	3	..	..
Ludhiana	September . . .	38	32	..
"	October . . .	12	19	15
"	November . . .	..	..	47

From the four plants dealt with so far, only *E. insulana* has been bred. All these except *Hibiscus cannabinus* are wild plants.

Hibiscus esculentus (*bhindi*). It is a cultivated plant which is extensively grown as a vegetable crop, particularly in the vicinity of towns. It may be sown at any time from March to June and bears flower-buds and pods mainly from June to November. Table V gives the total number of moths bred and the proportion of the species emerging during different months.

It is interesting to find that all the three species subsist on this plant. *E. fabia* was very common from July to October and its proportion was generally over 70 per cent and at times even cent per cent; *E. insulana*, on the other hand, was very common

during May, June and November. *E. cupreoviridis* was bred only during July, August and September mainly at Lyallpur and Sialkot, and its proportion was generally less than 10 per cent. Only once it was bred at Ludhiana. It was not obtained from Rohtak and Multan.

It has been shown [Haroon Khan, 1944] that the population of *E. fabia* rises suddenly on cotton in some parts of the Punjab during July and falls down rapidly in November. It is interesting to observe similar fluctuations in the population of this species on *bhindi* also. Further, *bhindi*

TABLE V

Number and proportion of *Earias* species on *Hibiscus esculentus*

Period	Rohtak				Ludhiana				Sialkot				Lyallpur				Multan			
	No. of moths	Percentage of <i>insulana</i>	Percentage of <i>fabia</i>	Percentage of <i>cupreoviridis</i>	No. of moths	Percentage of <i>insulana</i>	Percentage of <i>fabia</i>	Percentage of <i>cupreoviridis</i>	No. of moths	Percentage of <i>insulana</i>	Percentage of <i>fabia</i>	Percentage of <i>cupreoviridis</i>	No. of moths	Percentage of <i>insulana</i>	Percentage of <i>fabia</i>	Percentage of <i>cupreoviridis</i>	No. of moths	Percentage of <i>insulana</i>	Percentage of <i>fabia</i>	Percentage of <i>cupreoviridis</i>
1934																				
June	..	..	..	..	17	100	0	0	..	..	..	..	..	..	..	..	..	..	..	..
July	..	..	..	..	6	83.3	16.7	0	..	..	..	..	..	..	..	..	..	..	..	..
August	..	..	..	..	231	0	100	0	..	..	..	..	..	..	..	..	..	..	..	..
September	..	..	..	..	85	1.2	98.8	0	..	..	..	..	..	..	..	..	..	..	..	..
October	..	..	..	..	67	100	0	0	86	32.5	67.5	0	..	..	..	..	..	..	..	..
November	..	..	..	..	18	94.4	5.6	0	..	..	..	..	..	..	..	..	..	..	..	..
December	..	..	..	..	7	100	0	0	..	..	..	..	..	..	..	..	..	..	..	..
1935																				
July	..	..	..	..	125	100	0	0	8	100	0	0	..	..	..	..	..	..	..	..
August	..	..	..	..	187	99.5	0.5	0	10	60	0	40	..	..	..	..	..	..	..	..
September	..	..	..	..	230	30	70	0	..	..	..	..	61	11.5	80.3	8.2	11	9.9	9.1	0
October	34	8.8	91.2	0	281	4.9	90.8	4.3	87	13.8	67.8	18.4	310	4.5	94.2	1.3	7	28.6	71.4	0
November	38	10.5	89.5	0	228	8.3	91.7	0	80	3.8	88.7	7.5	350	1.1	98.9	0	78	15.4	84.6	0
December	21	9.5	90.5	0	107	92.5	7.5	0	20	60	40	0	191	41.4	58.6	0	9	11.1	88.9	0
January	..	..	..	..	13	100	0	0	11	100	0	0	41	82.9	17.1	0	19	89.5	10.5	0
February	..	..	..	..	..	..	..	..	10	100	0	0	..	..	..	..	..	..	..	..
1936																				
July	..	..	..	..	11	100	0	0	..	..	..	..	24	91.7	8.3	0	..	..	..	..
August	..	..	..	..	32	100	0	0	..	..	..	..	95	59.1	39.8	1.1	..	..	..	..
September	..	..	..	..	204	0.5	99.5	0	31	0	93.5	6.5	88	7.9	85.3	6.8	46	10.9	89.1	0
October	43	16.3	83.7	0	102	0	100	0	90	0	86.7	13.3	289	0	99.3	0.7	50	2.0	98.0	0
November	25	12.0	88.0	0	36	0	100	0	62	0	96.8	3.2	237	1.3	98.7	0	52	9.6	90.4	0
December	35	11.4	88.6	0	98	27.6	72.4	0	26	23.1	76.9	0	154	16.9	83.1	0	28	42.9	57.1	0
January	..	..	..	..	31	67.7	32.3	0	36	52.8	47.2	0	97	70.1	29.9	0	..	..	..	..

appears to be the most favourite host plant of *E. fabia*, because it was found in much larger numbers on this plant than on cotton or any other plant all over the province. *E. insulana*, on the other hand, was not common on *bhindi* during the period cotton was bearing large number of flower-buds and bolls—July to October, and the high proportion of this

species on *bhindi* during certain months was partly due to the scarcity of food on cotton and partly to the low population of *E. fabia* in the field.

The cultivation of *bhindi* as a trap crop around cotton fields has been recommended as a measure of control by a number of workers, Lefroy [1906], Fletcher and Misra [1919] and Jhaveri [1921].

Since only *E. fabia* is found on this host in appreciable numbers during the main cotton season, it is obvious that this measure will be effective only in places where *E. fabia* is the chief pest.

Althea rosea (Hollyhock). It is a cultivated and a common ornamental plant grown in gardens and parks, usually near the towns. It is sown at any time from October to January and bears flower-buds and pods mainly from March to June.

TABLE VI
Number of *Earias* species bred from *Althea rosea*

Period	Rohtak				Ludhiana				Sialkot			
	No. of moths	No. of <i>insulana</i>	No. of <i>fabia</i>	No. of <i>cupreoviridis</i>	No. of moths	No. of <i>insulana</i>	No. of <i>fabia</i>	No. of <i>cupreoviridis</i>	No. of moths	No. of <i>insulana</i>	No. of <i>fabia</i>	No. of <i>cupreoviridis</i>
1934												
April	..	..	..	..	..	..	..	..	10	10	0	0
May	..	..	..	..	31	31	0	0	5	5	0	0
June	..	..	..	..	23	21	0	2	1	1	0	0
August	..	..	..	..	3	0	3	0	..	..	..	..
1935												
March	127	127	0	0	28	28	0	0	..	..	..	..
April	63	62	1	0	15	15	0	0	..	..	..	..
May	103	100	3	0	192	183	0	9	..	..	..	..
June	28	28	0	0	29	29	0	0	..	..	..	..
1936												
April	48	44	4	0	18	18	0	0	..	..	..	..
May	52	47	5	0	19	18	0	1	..	..	..	..

From the above it is evident that this plant harbours all the three species. Of the 785 moths bred, 12 were *E. cupreoviridis*, 16 *E. fabia* and the rest *E. insulana*. Fletcher [1917] states that this is not a favourite food plant of *E. fabia* and *E. insulana*. His finding seems to be substantiated by the fact that a number of small caterpillars were found dead in the gummy substance coming out of the wounds caused by their feeding.

It is interesting to find that the two cultivated plants, *Hibiscus esculentus* and *Althea rosea*, harbour all the three species. Does it indicate that through domestication they have lost characters which make certain other plants distasteful to some of the species of *Earias*?

Malvestrum tricuspidatum. It is a perennial weed, commonly found in moist and shady places. It usually bears flower-buds and pods from May to November, but near some well irrigated plots the plant may bear them all the year round.

Of the 907 moths bred, 591 or just over 65 per cent moths were *E. cupreoviridis* and the rest *E. insulana*. From July to October, majority of the moths that emerged were *E. cupreoviridis*, while during other months *E. insulana* exceeded in number.

Sida cordifolia. It is a small perennial weed found in waste lands, and bears flower-buds and pods generally from July to November. It is found mainly in the South-eastern Punjab.

TABLE VII*

Number of *Earias* species bred from *Malvestrum tricuspidatum*

Period	Rohtak			Sialkot			Lyallpur		
	No. of moths	No. of <i>insulana</i>	No. of <i>cupreoviridis</i>	No. of moths	No. of <i>insulana</i>	No. of <i>cupreoviridis</i>	No. of moths	No. of <i>insulana</i>	No. of <i>cupreoviridis</i>
1935									
May	..	..	..	..	..	..	43	39	4
June	..	..	..	..	..	..	10	10	0
July	..	..	..	4	0	4	87	7	80
August	..	..	..	3	0	3	49	0	49
September	..	..	..	11	1	10	24	0	24
October	..	..	..	5	5	0	..	..	..
November	..	..	..	..	..	..	..	..	..
1936									
January	..	..	..	15	15	0	..	..	..
April	..	..	..	..	..	..	..	..	..
May	69	67	2	13	13	0	..	..	..
June	25	13	12	6	6	0	143	31	112
July	17	2	15	..	..	..	37	0	37
August	26	0	26	46	0	46	45	0	45
September	29	0	29	5	2	3	38	6	32
October	4	0	4	5	3	2	53	6	47
November	21	21	0	3	0	3	4	2	2
December	42	42	0	1	1	0	..	..	..

*9 moths, all *E. insulana*, were bred at Multan during October-November, 193515 moths, all *E. insulana*, were bred at Ludhiana in September-October, 1936

TABLE VIII

Number of *Earias* species on *Sida cordifolia*

Station	Period	No. of moths	No. of <i>insulana</i>	No. of <i>cupreoviridis</i>
Ludhiana	September 1934	20	2	18
"	October 1934	13	1	12
"	May 1935	2	0	2
"	August-September 1935	3	1	2
"	October 1935	40	3	37
"	November 1935	5	0	5
"	September 1936	67	0	67
Rohtak	July 1936	85	0	85
"	August 1936	115	0	115
"	September 1936	12	0	12
Sialkot	October-November 1936	20	0	20

Mostly *E. cupreoviridis* moths were bred from this host; of 382 moths that emerged only 7 were *E. insulana*.

These two wild plants, *M. tricuspidatum* and *S. cordifolia*, are favourite hosts of *E. cupreoviridis*.

SUMMARY

Althea rosea and *Hibiscus esculentus*, both cultivated plants, are common hosts of all the three species of *Earias*.

E. cupreoviridis is mainly found on *Malvestrum tricuspidatum* and *Sida cordifolia*, both wild plants, and only occasionally on the two common host plants; *E. fabia* is mainly found on *Hibiscus esculentus* and to a small extent on *Althea rosea*, while *E. insulana* is found on almost all the host plants, but very likely prefers *Abutilon indicum* most.

E. cupreoviridis is not common in the Punjab; nor are its host plants common.

E. fabia is very common from July to October when its favourite food plant—*bhindi*—is bearing large number of pods. It is possible that it attacks cotton when its population has increased considerably on *bhindi*.

E. insulana is the commonest species in the province. It seems to be least specialized with regard to its host plants and its wide distribution in many countries in Africa and Western Asia may partly be on account of its adaptability to subsist on several hosts.

ACKNOWLEDGEMENTS

This investigation was a part of the Punjab Bollworm Scheme, financed by the Indian Central Cotton Committee, and we express our gratitude to the Committee. We record our grateful thanks to Mian Afzal Husain, under whose guidance this work was conducted, for his constant help, and for going through the manuscript and making valuable suggestions. We are also indebted to Messrs P.M. Verma and Ghulamullah for their help in the collection of data at Rohtak and Multan, and to the Imperial Entomologist for giving facilities in the final revision of this paper.

REFERENCES

- Afzal Husain, M. (1925). Report of the Entomologist Government, Punjab: *Ann. Rep. Dept. Agric., Punjab*, 1923-24, pt. III, 55-90
- (1929). Report of the Entomologist, Government, Punjab. *Ann. Rep. Dept. Agric., Punjab*, 1927-28, pt. II, 55-79
- Bedford, H. W. (1931). The weed 'Hambuk' and the part it plays in the conservation of parasites of bollworms. *Wellcome Trop. Res. Lab. Ent. Sec. Bull.* **34**, 39-79
- Chopra, R.L. (1928). Report of the Entomologist, Government, Punjab. *Ann. Rep. Dept. Agric., Punjab*, 1925-26, Pt. III, 67-125
- Deshpande, B.P. and Nadkarny, N.T. (1936). Spotted bollworms of cotton in the South Gujrat. *Imp. Coun. Agric. Res. Sci. Monogr.* **10**, 1-208
- Dudgeon, G.C. (1916). The Bollworm in Egypt. *Trans. Third International Congress Trop. Agric.*, 1-34, (Abstr. R.A.E. **4**, Series A, 402-3)
- Fletcher, T.B. (1917). Insect pests of cotton. *Rep. Proc. Second Ent. Meeting, Pusa*, 104-32
- (1919). Annotated list of Indian Crop Pests. *Rep. Proc. Third Ent. Meeting, Pusa*, I, 78
- and Misra, C.S. (1919). Cotton Bollworms in India. *Rep. Proc. Third Ent. Meeting, Pusa*, II, 443-72
- Gough, L.H. (1919). The Pink Bollworm in Egypt. *Rep. Proc. Third Ent. Meeting, Pusa* II, 479-80
- Haroon Khan, M. (1944). The relative abundance of *E. fabia* and *E. insulana* on cotton. *Indian J. Ent.* **6**, 15-27
- Jhaveri, N.T. (1921). Cotton Bollworms in India. *Rep. Proc. Fourth Ent. Meeting, Pusa*, 93-5
- King, H.H. (1918). The control of insect pests of cotton. *Wellcome Trop. Res. Lab. Ent. Sec. Bull.* **9**, 1-4
- Lefroy, H.M. (1906). *Indian Insect Pests*, 89-92
- (1909). *Indian Insect Life*, 456
- Li (Feug-Swen) (1937). Cotton pests in China. *Ent. & Phytopath.* **5**, 1, 2-10 (Abstr. R.A.E. **25**, Series A, 791)
- Richards, P.B. (1924). The control of cotton pests in North India. *Agric. J. India* **19**, 568-78

RESEARCH NOTE

A PRELIMINARY NOTE ON THE DISTRIBUTION OF FRUIT PULP IN CITRUS SQUASHES

Y. GIRDHARI LAL, PH.D. (LOND.), D.I.C., Fruit Biochemist, and NAGINA LAL JAIN, M.Sc. (TECH.),
Research Assistant, Fruit Products Laboratory, Lyallpur

(Received for publication on 31 August 1944)

It is a matter of common experience that fruit pulp in bottled citrus squashes (foreign or local made) partly settles at the bottom and partly floats on top. When the pulp floats on the top it presents a very unsightly and clumpy appearance and reduces considerably the market value of the product. This difficulty has been sought to be remedied by manufacturers in this country, particularly in the case of lime squash, in which most of the pulp in the form of a thick oily emulsion has a tendency to float on the top of the bottled product. A detailed study of the problem was undertaken by the authors under the Special Fruit and Vegetable Preservation Scheme, Lyallpur, financed by the Imperial Council of Agricultural Research.

Preliminary experiments were carried out in the case of lime squash. In all, 46 different sets of this squash were prepared (according to the usual formula, 1 lb. juice, $1\frac{1}{4}$ lb. sugar and $\frac{3}{4}$ lb. water) with the following treatments:

1. Different methods for extraction of juice, *viz.* (a) with hand juice extractors and, (b) in a basket press.
2. Thorough shaking of the squash and of the juice before preparing squash, to break the colloidal nature of the pulp.
3. Shaking of squash and of juice before preparing squash for 24 hours in a mechanical shaker.
4. Juice passed through fine holes under vacuum before preparing squash.
5. Heating the juice at 60°C. for 5-15 min. before preparing squash.
6. Heating the prepared squash at 60°C. for 15 min.

7. 'Flash' pasteurization of juice before preparing squash.

8. Placing the bottles of squash lengthwise and upside down.

9. Centrifuging the juice in a tube centrifuge, before preparing squash.

10. Addition of the following chemicals (with the range given in each case) to the juice before preparing squash:

(a) 0.025 to 0.1 per cent common salt, (b) 0.025 per cent calcium chloride, (c) 0.05 to 0.5 per cent gum tragacanth, (d) 0.05 to 0.5 per cent solid pectin, (e) 0.5 to 1.0 per cent gum acacia, (f) 12.5 to 75.0 per cent by weight of a water extract prepared from the rag of limes left after juice extraction. (An equal amount of water was reduced in the formula in this case.)

Out of the above treatments, the heating of the prepared squash at 60°C., addition of substances like gum tragacanth, pectin, common salt, gum acacia and rag extract separately to the juice (before preparing squash), made the pulp settle at the bottom. By an addition of varying amounts of rag extract, it has also been possible to keep the entire pulp uniformly distributed throughout the product, a procedure which yields a cloudy squash in which no separation of pulp takes place. None of the above treatments imparts any undesirable taste or flavour to the product.

Experiments are also now under way, in which the results of the above investigation are being extended to other citrus squashes like those of grapefruit, lemon, Malta and *Sangtra*. Encouraging results have been obtained in this connection. The detailed results of the investigation will be published as soon as storage trials of these products have been completed.

PLANT QUARANTINE NOTIFICATIONS

The following Quarantine regulations have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi.

NOTICE No. 2 OF 1945

1. Mexican Fruitfly Quarantine—B.E.P.Q. 64 issued by the United States Department of Agriculture.
2. Plant Quarantine Import Restrictions of the Presidency of Antigua, B.W.I. dated 20 January 1945, issued by the United States Department of Agriculture.

NOTICE No. 3 OF 1945

1. Service and Regulatory Announcements July-September 1944 issued by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.
2. White-Fringed Beetle Quarantine (Quarantine No. 72) issued by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.
3. Bureau of Entomology and Plant Quarantine—479, Supplement No. 1, Revised, issued by the United States Department of Agriculture.

Editorial Committee

- | | |
|---|---|
| <p>H. R. STEWART, C.I.E., M.Sc.,
F.R.C.Sc.I., D.I.C., N.D.A., I.A.S.,
<i>Vice-Chairman, Imperial Council of
Agricultural Research</i></p> <p>MAJOR G. WILLIAMSON, O.B.E.,
M.R.C.V.S., D.V.S.M., <i>Animal
Husbandry Commissioner with the
Government of India</i></p> <p>HEM SINGH PRUTHI, M.Sc. (Ph.),
Ph.D. and Sc.D. (Cantab.),
F.R.A.S.B., F.N.I., <i>Director, Impe-
rial Agricultural Research Institute,
New Delhi</i></p> <p>F. C. MINETT, C.I.E., D.Sc., M.R.C.V.S.;
<i>Director, Imperial Veterinary Re-
search Institute, Mukteswar</i></p> <p>ZAL R. KOTHAVALLA, B.Ag., B.Sc.,
N.D.D., <i>Dairy Development Adviser
to the Government of India, New
Delhi</i></p> | <p>K. C. SEN, D.Sc., <i>Director of Dairy Re-
search, Bangalore</i></p> <p>J. N. MUKHERJEE, C.B.E., D.Sc., <i>Ghose
Professor of Chemistry, University
College of Science and Technology,
Calcutta</i></p> <p>BIRBAL SAHNI, M.A., Sc.D. (Cantab.),
D.Sc. (Lond.), F.R.S., <i>Professor of
Botany, Lucknow University,
Lucknow</i></p> <p>JAMES N. WARNER, M.Sc., <i>Professor of
Animal Husbandry and Dairying,
Allahabad Agricultural Institute,
Allahabad</i></p> <p>S. KRISHNA, C.I.E., Ph.D., D.Sc., F.I.C.,
<i>Biochemist, Forest Research Institute,
Dehra Dun</i></p> <p>RAI SAHIB S. C. SARKAR, B.A., <i>Secretary,
Imperial Council of Agricultural
Research</i></p> |
|---|---|

Editor

U. N. CHATTERJI, D. Phil., D.Sc., F.N.A.Sc.

The Editorial Committee, in its work of examining papers received for publication, is assisted in an honorary capacity by a large number of scientists working in various parts of India.

Editorial communications including books and periodicals for review should

be addressed to the Secretary, Imperial Council of Agricultural Research, Publication Section, New Delhi.

Communications regarding subscription and advertisements should be addressed to the Manager of Publications, Civil Lines, Delhi.

Instructions to Authors

Articles intended for the *Indian Journal of Agricultural Science* should be accompanied by short popular abstracts of about 300 words each.

In the case of botanical and zoological names the International Rules of Botanical Nomenclature and the International Rules of Zoological Nomenclature should be followed.

References to literature, arranged alphabetically according to author's names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, title of the article, the abbreviated title of the publication, volume and page. In the text, the reference should be indicated by the author's name, followed by the year of publication enclosed in brackets; when the author's name occurs in the text, the

year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and the number quoted after year both in the text and in the collected references.

If a paper has not been seen in original it is safe to state 'original not seen'.

Sources of information should be specifically acknowledged.

As the format of the journals has been standardized, the size adopted being crown quarto (about 7½ in. × 9½ in. cut), no text-figure, when printed, should exceed 4½ in. × 5 in. Figures for plates should be so planned as to fill a crown quarto plate, the maximum space available for figures being 5½ in. × 8 in. exclusive of that for letterpress printing.

Copies of detailed instructions can be had from the Secretary, Imperial Council of Agricultural Research, New Delhi.